

**Describing and understanding host-pathogen community interaction at the
wildlife/domestic interface**

By

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Describing and understanding host-pathogen community interaction at the
wildlife/domestic interface

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Abstract

In this thesis, I investigated the relationship between host and pathogen in multi-host and multi-pathogen systems at the interface between wildlife and domestic species. The term “epidemiological interaction” was central to my thesis, and was defined as “any ecological interaction between two host populations resulting in the transmission of one or more pathogen”. Epidemiological interactions are related to the processes of transmission between hosts and I investigated how these epidemiological interactions between different host populations could be investigated in a given ecosystem. I developed two research frameworks to estimate these epidemiological interactions: 1) an *a priori* approach based on the host data and assuming that the mobility of hosts and the resulting contacts between host populations would be crucial factors influencing the epidemiological interactions; 2) an *a posteriori* approach based on the pathogen data, assuming that epidemiological pathways previously used by some pathogen species can be used in the future by other pathogens. The animal-pathogen model used to test the first approach was the bird-avian influenza viruses’ model. Longitudinal counting and sampling protocols of domestic and wild birds over two years were used to analyse community composition and abundance of hosts to compare with the prevalence of avian influenza viruses. I could, for the first time, show a persistence of low pathogenic avian influenza strains in an African ecosystem, and investigate the relationships with both the potential maintenance hosts (Afro-tropical ducks and resident species) and hosts that introduced the virus into the system from Europe or Asia (paleartic migrants). With the estimation of epidemiological interaction using host community data, I estimated the contact rate between wild and domestic avian compartments (intensive poultry, backyard and farmed ostrich compartments) and assigned a risk to this interaction based on dynamic and non-

dynamic factors for each bird species. This approach highlights the species or seasons at risk for the domestic compartments (or for the wild bird compartments depending on the perspective) in order to orientate surveillance or control options. This type of data and framework can also be used in mechanistic modelling to predict the spread of a pathogen after its introduction in one compartment. I tested the host approach in a broader dataset at the Southern African region level with similar counting and sampling database in multiple study sites, showing that the variability of host communities across the region could explain the variability of pathogen detection (however, finding a causal relationship was impossible). Finally, I theoretically developed the pathogen approach by combining tools used in parasite community ecology, molecular epidemiology and social network analysis and gave a theoretical example using a rodent and human macro and microparasite dataset.

This thesis has explored the field of transmission ecology and offered ways to quantify the processes of transmission between host populations. Theoretically, I have developed a fundamental reflexion around epidemiological interactions and formulated hypotheses on their potential for being independent of the parasite species. Practically, I have developed tools to provide information for decision-making in order to improve efficiency of surveillance and control programmes at the wildlife/domestic interface particularly adapted to detect emerging infectious disease spill-over process.

I, Alexandre Caron, declare that the thesis/dissertation, which I hereby submit for the degree of Doctor of Philosophy at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

SIGNATURE:

DATE: 15 January 2011

Summary

Describing and understanding host-pathogen community interaction at the
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By

Alexandre Caron

Doctor of Philosophy in

Department of Zoology and Entomology

University of Pretoria

I have defined the concept of epidemiological interaction (EI) between two host populations (**Chapter One** is a theoretical introduction). Then I have presented two approaches to estimate these EIs. The first one, described in **Chapter Two**, assumes that the movements of hosts and the contacts induced by this mobility will contribute to estimating EIs. The second one (**Chapter Seven**) assumes that there is a limited quantity of potential transmission pathways between two host populations and that past occurrence of disease transmission can estimate future occurrence of pathogen spill-over or disease emergence.

In this thesis, I have developed mostly the first approach. Using the model of wild and domestic avian communities in a Zimbabwean wetland, I have gathered longitudinal ecological and epidemiological data to provide information about Avian Influenza viruses (AIV) in a multi-host system. In **Chapter Three**, I have explored how a host community approach can help defining a risk season, and risk species for the epidemiological cycle (reservoir, spreader, maintenance host) and at the wildlife/domestic interface. This has been used to identify potential bridge species able to spread the pathogen from a source host population to a target host population. In **Chapter Four**, I suggest for the first time a yearly persistence of AIV in an African ecosystem based on multi-species data and by exploring potential mechanisms for this persistence. In **Chapter Five**, I show how ecological and epidemiological data can be integrated in a risk analysis to estimate the risk of pathogen spread through wild birds and present a framework for animal health services to increase the efficiency of their surveillance at the wildlife/domestic interface. **Chapter Six** explores the complexity of predicting AIV circulation in wild bird communities at the southern African level, based on a regional dataset and presenting an epidemiological functional group approach (borrowed from the functional group concept in community ecology). Articles included as appendices show how this work is integrated in a broader framework of research on the ecology of AIV in waterbirds (**Appendix One, Two, Four & Five**).

In parallel, the same approach was used in wild/domestic ungulate community in the South-East Lowveld of Zimbabwe, exploring transmission pathways for bovine tuberculosis and other important diseases (**Appendix Three** & on-going manuscripts). The juxtaposition of these models highlights the process-centred approach that I have focused on instead of a host- or pathogen-centred approach: I am (in collaboration with my colleagues) exploring and testing the same hypotheses in the two models.

In **Chapter Seven** (and **Appendix Six**), I present the pathogen approach that I have developed, presenting the conceptual and operational framework to identify the most likely transmission pathways at the community level using social network analysis. I use an example on rodent community and their parasite community in South East Asia (Box in **Chapter Seven**).

Finally, in **Chapter 8**, I synthesise the finding of this thesis, try to provide a research framework for future research and discuss future breakthrough in technology which will provide major advances in the ecology of disease transmission.



Note on the text

Each chapter is set out in the style of the journal to which it has or will be submitted. Consequently there is some repetition and stylistic differences in each of the chapters. In addition, other authors are included in the paper reference. However, for each chapter, my input was the greatest. I planned research, undertook the field work, analysed the data and wrote the manuscripts. I was helped by my co-authors. Elissa Cameron, Michel de Garine-Wichatitsky, David Cumming and Serge Morand were my supervisors.

Acknowledgements

Starting from the beginning, I would like to thank my parents and my sister for giving me the opportunity to grow and then study in a perfect family environment. My parents gave me the education and the will to have goals to achieve and dreams to fulfill. This thesis is the completion of both. A tous les trois, mille mercis pour votre aide, soutien et amour.

If I engaged in a Phd thesis at some point of my career, it is because someone advised me to do so and felt that I could become a researcher. I did not agree at that time but he was right. I would like therefore to thank François Monicat, former head of the research unit I belong to who gave me that piece of advice at the right time. In addition, he put me in a professional position in Zimbabwe, where I could build a proper framework for this thesis and implement it with time and means.

On the (long) way to become a researcher (and I still have some way to go), I met Michel de Garine-Wichatitsky, a “real” researcher, with the right spirit and skills to train me. We have shared the same office for more than four years now and this type of relationship can only evolved in two ways: we took the right one. Fortunately, he was patient and diplomat enough to accept my numerous questions about protocols, statistical issues and relevance of my thoughts and writing. He had a huge impact on this thesis and I hope we will be able to share this professional experience for many more years. I am honored to be able to benefit from his experience and friendship.

This PhD thesis cannot be a one-person achievement, even if I am responsible for all its imperfections. It represents more than four years of collaboration with researchers and non-researchers. First of all, I would like to thank my supervisor, Elissa Cameron who accepted me as a PhD student based on trust and a few ideas on a piece of paper. Then, Elissa

has always been supportive of my project, ready to help me on manuscripts and to reformulate clearly my thoughts. I only regret that I could not show her my study sites in Zimbabwe due to time and agenda constraints. My co-supervisors, Michel de Garine-Wichatitsky, Serge Morand and David Cumming helped me a lot to give this thesis a proper framework and to bring a necessary eagle-view to my different works. They have spent much of their precious time for me and I will always be thankful for that. This thesis is also a tremendous collaborative study involving researchers from Europe, Africa and Asia. I would like to highlight the good spirit that prevails in the AGIRs research unit of Cirad in which I was hosted all these years. I met incredible researchers and friends and only hope to justify my place in their team in the future. I had very good collaborations with various institutes and research unit. First in Zimbabwe, I was kindly hosted by the research platform RP-PCP (Production and Conservation in Partnership), a collaborative experience between University of Zimbabwe, the National University of Sciences and Technology (NUST), Cirad and CNRS. Then through various projects, I had the chance to work with the Mammal Research Institute of course, but also the ARC-OVI (Agricultural Research Council - Onderstepoort Veterinary Institute) research laboratory in Pretoria, and the Fitz-Patrick Institute in Cape Town.

A part from methodology and theoretical science, field activities were an important part of this thesis. Through the year, I was helped by many skilled and motivated individuals that I cannot all cite. The Cirad team in the Zimbabwean office provided me with a good and supportive environment to develop my research. I will also thank the Veterinary Services of Zimbabwe and the Park and Wildlife Management Authority of Zimbabwe which provided me with access to their fields, supportive staff and various technical and administrative supports. Despite a very difficult socio-economic context in Zimbabwe at the beginning of this thesis, they always answered present to my requests. Finally, I was closely helped by a

research assistant, Ngoni Chiweshe, a skilled ornithologist who became an efficient bird sampler and a good database handler!

Research can only be implemented if donors support ideas and projects. The French Ministry of Foreign Affairs (MAEE) has funded the Mesures d'Urgence and GRIPAVI projects from 2007 to end of 2011. The French Embassy in Zimbabwe has also always been supportive of Cirad activities in the country and helped many Zimbabwean students to achieve graduate and post-graduate degrees by completing projects related to this thesis. FAO (Food and Agriculture Organisation), the European Union office in Zimbabwe (PARSEL project) were also supportive of this project.

Finally this thesis, despite all the collaboration cited, is a single individual responsibility. If I can pretend to be the one who completed this work, it can only be due to my personal well-being. Friends in France, Zimbabwe, South Africa and elsewhere are crucial to keep a personality alive. But I will not be myself without Carole. She left her life in France to follow me in an unknown continent, to build a complete new life in a new environment and at the same time, supporting my absences and my doubts. She managed to create a new family environment around us, first together, and then with our wonderful daughters, Eva and Noémie who landed in our life during this thesis. This was the environment I needed to complete this thesis.

These five years in Zimbabwe would mean a lot in my life, forever.

List of Contents

Abstract.....	v
Summary.....	ix
Note on the text.....	xiii
Acknowledgments.....	xv
List of Contents.....	xix
List of Tables.....	xxvii
Chapter Tables.....	xxvii
Appendix Tables.....	xxix
List of Figures and Boxes.....	xxxiii
Chapter Figures and Boxes.....	xxxiii
Appendix Figures and Boxes.....	xxxv

Chapters

Chapter One.....	1
<i>General Introduction</i>	
Thesis Outline.....	5
Literature Cited.....	12
 Chapter Two.....	 17
<i>Evolutionary Biology, Community Ecology and Avian Influenza Research</i>	
Background and context.....	18
Current understanding of AIV epidemiology.....	19
HPAI H5N1 epidemiology.....	20
Avian host ecology.....	22
Role of interactions of hosts between compartments in AIV evolution.....	23
Implication of evolutionary biology and community ecology for AIV research.....	30

Conclusion: describing the pathogen risk.....	32
Literature cited.....	34
Chapter Three.....	45
<i>Estimating Dynamic Risk Factors for Pathogen Transmission using Community-level Bird Census Data at the Wildlife/Domestic Interface</i>	
Introduction.....	46
Methods.....	49
Study site.....	49
Hazard identification.....	50
Community composition.....	51
Ecological risk factors (RFs)	52
Introduction and maintenance risk (IR and MR) in waterfowl community.....	55
Quantifying epidemiological interaction (domestic risk – DR) and their risks.....	55
Results.....	56
Dynamics of waterfowl community.....	56
Dynamics of the domestic communities.....	56
Patterns of IR and MR in the waterfowl compartment and relation to the RFs.....	57
Variation in DR for the three domestic compartments.....	62
Discussion.....	66
On the use of dynamic risk factors.....	66
Domestic risk (DR) between waterfowl and domestic compartments.....	71
Validating the model and testing the bridge species hypothesis.....	73
Conclusions.....	75
Literature cited.....	76
Chapter Four.....	85
<i>Persistence of Low Pathogenic Avian Influenza Virus in Waterfowl in an African Ecosystem</i>	
Introduction.....	86
Methods.....	86

Results.....	89
Discussion.....	93
Conclusion.....	98
Literature cited.....	99

Chapter Five.....103

Risk of diffusion of a Highly Pathogenic Avian Influenza virus between wild and domestic avian compartments through wild birds in Zimbabwe

Introduction.....	104
Methods.....	106
Study site.....	106
Counting protocols.....	108
Estimation of epidemiological interactions.....	108
Risk analysis approach.....	111
Hazard identification.....	112
Framing the risk question.....	112
Release assessment.....	112
Exposure assessment.....	112
Consequence assessment.....	112
Results.....	115
Risk assessment.....	115
Release assessment.....	115
Exposure assessment.....	118
Discussion.....	134
Exposure assessment.....	134
Consequence assessment.....	136
Risk management.....	138
Literature cited.....	142

Chapter Six.....147

Exploring the relation between avian communities and AIV ecology in Southern Africa using the concept of epidemiological functional groups

Introduction.....	148
Methods.....	153
Study site.....	153
Baseline data.....	153
Data analysis.....	154
First step: comparison of bird communities between sites.....	154
Second step: comparison of birds sampled and birds observed.....	154
Third step: epidemiological functional group approach.....	154
Results.....	156
First step: comparison of waterfowl communities between sites.....	156
Second step: comparison of bird sampled and bird observed.....	157
Third step: epidemiological functional group approach.....	158
Discussion.....	164
First step: comparison of waterfowl communities between sites.....	164
Second step: comparison of bird sampled and bird observed.....	165
Third step: AIV prevalence estimation using the EFG approach.....	166
Literature cited.....	171

Chapter Seven.....177

Ecology of disease transmission in multi-host systems

Introduction.....	178
Critical advances in ecology and epidemiology.....	178
Conceptual and operational framework.....	182
Scope and limitations of the approach.....	193
EID at the wildlife/domestic/human interface.....	194
Literature cited.....	197

Chapter Eight.....	211
---------------------------	------------

General Conclusions

Literature cited.....	217
-----------------------	-----

Appendices

Appendix One.....	221
--------------------------	------------

Avian influenza A viruses in waterbirds in Africa

Introduction.....	222
The Study.....	222
Conclusions.....	228
Literature cited.....	230

Appendix Two.....	233
--------------------------	------------

Influenza Surveillance in Wild Birds in Eastern Europe, the Middle East, and Africa: Preliminary Results from an Ongoing FAO-led Survey

Introduction.....	234
Methods.....	235
Results.....	240
Discussion.....	244
Literature cited.....	247

Appendix Three.....	249
----------------------------	------------

Bovine tuberculosis in buffaloes, Southern Africa

To the Editor.....	250
Literature cited.....	253

Appendix Four.....	255
---------------------------	------------

Understanding the ecological drivers of avian influenza virus infection in wildfowl: a continental scale study across Africa

Introduction.....	256
Materials and Methods.....	263
Results.....	265
Discussion.....	268
Supporting information.....	277
Literature cited.....	279
 Appendix Five.....	 285
<i>The ecology of Influenza A viruses in wild birds in southern Africa</i>	
Introduction.....	286
Methods.....	287
Project design and field sites.....	287
Counting protocols.....	289
Capture and sampling protocols.....	289
Data analysis.....	291
Results.....	292
Discussion.....	311
Supporting information.....	319
Literature cited.....	320
 Appendix Six.....	 325
<i>Epidemiological interaction at the wildlife/livestock/human interface: can we anticipate emerging infectious diseases in their hotspots? A framework for understanding emerging diseases processes in their hotspots</i>	
Introduction.....	326
Methods.....	328
Estimating transmission rate for pathogen shared between host populations.....	328
EI network and selection of host and pathogen community to predict pathogen emergence....	330
Estimating epidemiological interactions using host data (<i>a priori</i> approach).....	335
Estimating epidemiological interactions using the pathogen level (<i>a posteriori</i> approach)....	337

Discussion.....	342
Conclusion.....	352
Literature cited.....	355



List of Tables

Chapters Tables

<u>Table 3.1</u>	53
------------------------	----

Risk factors (RFs) used in this study, their derivation, and the motivation for including them.

<u>Table 3.2</u>	58
------------------------	----

Justification and equations for introduction, maintenance and domestic risk.

<u>Table 3.3</u>	59
------------------------	----

Standard deviation for each risk factors and the species diversity and Spearman Rank Correlation Coefficient for each risk factors and the species diversity in relation to the global risk.

<u>Table 3.4</u>	63
------------------------	----

Twenty most important species influencing the risk in our model ranked per Risk Factor (decreasing ranking) and sum across the 7 RFs values for the last column (RiskSum).

<u>Table 3.5</u>	68
------------------------	----

Twenty most important species influencing the maintenance risk in our model ranked per risk factor (decreasing ranking) and sum across the 5 RFs values for the last column (Maintenance Risk).

<u>Table 3.6</u>	72
------------------------	----

Most important families participating to the epidemiological interaction defined as domestic risk (DR) between the waterfowl and each of the 3 domestic compartments during peak risk period.

<u>Table 4.1</u>	90
------------------------	----

Sample size per session and proportion of swab sampled per order.

<u>Table 4.2</u>	91
<i>Prevalence, sample size for the entire birds sampled and only the ducks sampled compared between sessions when palearctic birds are present in the ecosystem and absent.</i>	
<u>Table 4.3</u>	92
<i>PCR positive swabs for Avian Influenza and hemagglutininase type when available.</i>	
<u>Table 5.1</u>	114
<i>Table of correspondence for the products of qualitative probability.</i>	
<u>Table 5.2</u>	116
<i>Emergence pathways identified in the four avian compartments of the ecosystem.</i>	
<u>Table 5.3</u>	121
<i>Means and standard error (Std. Error) of ($n_b * n_b'$) and of species richness (Sp. Rich.) for interaction between each pair of compartments.</i>	
<u>Table 5.4</u>	122
<i>Means and standard error of ($n_b * n_b'$) and of species richness (Sp. Rich.) for seasons and years.</i>	
<u>Table 5.5</u>	126
<i>Dominant species for each interaction sum for each session of the longitudinal protocol for each pair of compartments.</i>	
<u>Table 5.6</u>	128
<i>Dominant species for each interaction sum for each session of the intensive protocol for each pair of compartments.</i>	
<u>Table 5.7</u>	131
<i>Risk Table combining the risk of spread from the ostrich compartment.</i>	

<u>Table 5.8</u>	132
<i>Risk Table combining the risk of spread from the waterfowl compartment to the remaining two compartments</i>	
<u>Table 6.1</u>	159
<i>Indicators of waterfowl community diversity.</i>	
<u>Table 6.2</u>	161
<i>Prevalence (Prev) of AIV and confidence interval (CI, Lower and Upper boundaries) at 95% calculated for each site across the 12 sampling sessions at the community level and for each group of both EFs.</i>	
<u>Table 7.1</u>	181
<i>Properties of epidemiological interactions between two host populations and references (illustrative, not exhaustive) for methods potentially needed for epidemiological interaction networks.</i>	
<u>Table 7.2</u>	189
<i>Host and parasite species used.</i>	
<u>Table 7.3</u>	189
<i>Jaccard index for all pairs of host populations; habitat type for rodent species is mentioned: 1=in human settlements; 2=in rice fields; 3=in modified forest and ; 4=in primary forest; the number of parasite species (Nb of para) per host is also indicated.</i>	

Appendix Tables

<u>Table A1.1</u>	224
<i>Prevalence of influenza A virus in wild birds detected by RT-PCR.</i>	
<u>Table A1.2</u>	227
<i>RT-PCR based detection of influenza A virus in two wild ducks sampled in various surveyed countries.</i>	

<u>Table A2.1</u>	236
<i>Avian influenza surveillance campaign results in Eastern Europe, the Middle East, and Africa in early 2006.</i>	
<u>Table A2.2</u>	241
<i>Prevalence of low pathogenic avian influenza virus detected by RT-PCR in wild birds.</i>	
<u>Table A2.3</u>	243
<i>Virus subtypes isolated from the RT-PCR positive samples.</i>	
<u>Table A4.1</u>	259
<i>Summary of our current understanding of AIV transmission in wildfowl in relation to host ecology and environmental drivers.</i>	
<u>Table A5.1</u>	293
<i>Numbers of birds sampled for avian influenza, by family and by site. BAR, Barberspan; CHU, Chualu; MAS, Massingir; NGA, Ngami; STR, Strandfontein; ZW, Zimbabwe (Chivero and Manyame).</i>	
<u>Table A5.2</u>	296
<i>Numbers of birds that tested positive for avian influenza, by family and site.</i>	
<u>Table A5.3</u>	299
<i>Prevalence of avian influenza by avian family and by site over the period March 2007-April 2009.</i>	
<u>Table 5A.4</u>	303
<i>Information on viral strains and types.</i>	
<u>Table A5.5</u>	307
<i>Paleartic migrants observed during point counts.</i>	

<u>Table A6.1</u>	345
-------------------------	-----

Host and parasite species used.

<u>Table A6.2</u>	346
-------------------------	-----

Matrix of Jaccard index for all pairs of host populations.



List of Figures and Boxes

Chapters Figures & Boxes

<u>Figure 2.1</u>	25
<i>Conceptual research framework using community ecology and evolutionary biology to explore AIV ecology within their host in ecosystems.</i>	
<u>Figure 3.1</u>	60
<i>Waterfowl community abundance per family and species diversity across the 12 missions.</i>	
<u>Figure 3.2</u>	61
<i>Number of birds observed per family and species diversity in the three domestic compartments.</i>	
<u>Figure 3.3</u>	64
<i>Variation in the introduction risk (combined immigration and AIV risk related RFs) calculated for all AIV and for H5N1 in particular.</i>	
<u>Figure 3.4</u>	65
<i>Evolution of the maintenance risk (MR) and of each RFs included in the MR in the waterfowl compartment.</i>	
<u>Figure 3.5</u>	69
<i>Interaction Risk (DR) for each domestic compartment associated with introduction (IR) and maintenance risk (MR) for the waterfowl community.</i>	
<u>Figure 4.1</u>	88
<i>Counting and capture sites (red dots) in Manyame (left) and Chivero (right) lakes with location of the ecosystem in Zimbabwe and of Zimbabwe in Africa.</i>	

<u>Figure 4.2</u>	96
<i>(a) Birds counted per session; (b) birds captured per session; (c) global and duck prevalence per session with confidence interval.</i>	
<u>Figure 5.1</u>	107
<i>Conceptual representation of the study site using compartments.</i>	
<u>Figure 5.2</u>	110
<i>Schematic representation of the transmission of a HPAI virus from an infected to a naive bird compartment</i>	
<u>Figure 5.3</u>	117
<i>Population dynamics in the ostrich and waterfowl compartments on a bi-annual basis.</i>	
<u>Box 5.1</u>	119
<i>Additional information on the exposure assessment of intensive & backyard chicken, waterfowl and ostriches.</i>	
<u>Figure 5.4</u>	123
<i>Variation of the log of the interaction sum in the longitudinal protocol across the fourteen counting sessions.</i>	
<u>Figure 6.1</u>	151
<i>Epidemiological Functional Group 1.</i>	
<u>Figure 6.2</u>	152
<i>Epidemiological Functional Group 2.</i>	
<u>Figure 6.3</u>	160
<i>Community observed (left) and captured (right) in the three sites according to EF 1 & 2 groups.</i>	

<u>Figure 6.4</u>	162
-------------------------	-----

AIV Prevalence and confidence interval for the three sites including bird community composition.

<u>Box 7.1</u>	183
----------------------	-----

Epidemiological Functional Groups.

<u>Box 7.2</u>	188
----------------------	-----

Epidemiological Interaction Network for 14 rodent species and the human species.

<u>Figure 7.1</u>	190
-------------------------	-----

Epidemiological Interaction Network for 14 rodent species and the human species in the Southeast Asian ecosystems based on presence-absence data for 34 macroparasite species and 8 microparasite species.

Appendix Figures & Boxes

<u>Figure A1.1</u>	223
--------------------------	-----

Locations of sampling sites (or cluster of sites) in surveyed countries (dark grey) initially participating in the FAO's Technical Cooperation Programmes (light and dark grey).

<u>Figure A2.1</u>	238
--------------------------	-----

Concomitance in timing of field campaigns in surveyed countries (sampling periods distributed along a temporal axis) and first HPAI H5N1 reported outbreaks (OIE-World organisation for animal health notification reports) in the surveyed regions between January and May 2006.

<u>Figure A4.1</u>	261
--------------------------	-----

A. The timing and duration of rainfall in sub-Saharan Africa and the seasonal position of the Inter-Tropical Convergence Zone (ITCZ) (30); B. The two main migratory flyways of Eurasian wildfowl wintering in sub-Saharan Africa; C. Distribution range of Afro-tropical wildfowl over the African continent; D. Location of sampling sites presented in our study, also showing sample size ranges.

<u>Figure A4.2</u>	269
<i>Effect plots of the four ecological factors identified as influencing the variation of AIV prevalence in wildfowl in Afro-tropical regions. Plots of predicted prevalence (95% CI) were generated from the highest rank model presented in Table A4.S7. Data points are plotted as rug plots.</i>	
<u>Figure A5.1</u>	288
<i>Map of southern Africa showing sampling sites mentioned in this paper.</i>	
<u>Figure A5.2</u>	290
<i>Example of a walk-in trap used to catch ducks. In this picture, XX (L) and YY (R) capture Egyptian geese at Strandfontein.</i>	
<u>Figure A5.3</u>	312
<i>Prevalence by site and month across all captured birds.</i>	
<u>Figure A5.4</u>	313
<i>Relative abundance of (a) Anatidae, (b) Charadriidae, (c) Jacanidae and (d) Dendrocygnidae at each of the four sites from which we obtained AIV positive samples.</i>	
<u>Figure A5.5</u>	315
<i>Relative abundance of infected anatids per half-hour point count by site and month. Note how the impression given by this figure differs from that in Figures 2 and 3(a).</i>	
<u>Figure A5.6</u>	316
<i>Relative abundance of palearctic migrants per half-hour point count by site.</i>	
<u>Figure A6.1</u>	332
<i>a) Theoretical contact network including wild, domestic and human populations; in the remaining figures, the same network when the human population (b), a domestic population (c) or a flagship wildlife species (d) is considered as the target species.</i>	

<u>Figure A6.2</u>	333
--------------------------	-----

Three-step process to build a EI network and its schematic representation.

<u>Box A6.1</u>	344
-----------------------	-----

Epidemiological Interaction Network for 14 rodent species and the human species.

<u>Figure A6.3</u>	347
--------------------------	-----

Epidemiological Interaction Network for 14 rodent species and the human species in the Southeast Asian ecosystems based on presence-absence data for 34 macroparasite species and 8 microparasite species.



Chapter One: General introduction

Alexandre Caron



I built this thesis on the basis of two recent facts about disease ecology in animals and humans: the majority of emerging diseases involve more than one host and the majority of these hosts are wildlife hosts; the disease spill-over occurs often at the wildlife/domestic interface. My main research question was: how do we study disease ecology in multi-host systems at the wildlife/domestic interface? Which tools integrating epidemiology (which is, to a certain extent, a subset of ecology) and ecology can we develop to adapt health surveillance and control to multi-host systems? Are there common properties in the ecology of disease transmission that we can estimate and use for disease control and surveillance? I develop the argumentation justifying this approach in this chapter and present an outline of this thesis.

The struggle against infectious diseases has a universal cost for living organisms. Parasites species represent between 10 and 50% of known living organisms depending on the definition one applies (Poulin and Morand 2000). Despite this reality, the ecological impact of parasitism on organisms and ecosystems has received little attention in the literature with dedicated studies on the matter blooming only recently (Hudson 2002). The ecology of parasites is of particular interest in the study of ecological interactions (since the parasite depends on one or more hosts for the completion of their life cycle) and because their role in ecosystems is poorly understood (Hudson et al. 2006). If the question of their importance is not in question in ecology, their impact on “important” species from an anthropological perspective makes some of them unwanted or in need for control. They have important implications for human health, domestic animal health, a key component of human food production systems and also wildlife health for direct conservation purposes or for ecosystem health or services. Humans, positioned *de facto* as nature managers, need to deal with the infectious diseases of those species they consider to be important. Understanding how some pathogens kill and others influence their hosts, how they are transmitted and how we can interfere with these processes for managing or controlling the disease is therefore

fundamental. This thesis aims at increasing theoretically our knowledge about the ecology of parasites in their hosts and improving practically our surveillance and control options.

The genetic and social evolution of the human species has produced a succession of stages defining our relationship with pathogens, sometime inducing huge impacts on the population dynamics of our own species and often initiated by crucial advances in technology or societies (Barrett et al. 1998). The plague epidemic in Europe in the XIVth century has been linked to the emergence of urban centres connected to each other by more efficient terrestrial or maritime routes (Gage and Kosoy 2004). The recent globalisation in terms of movements of goods, animals, people and indirectly pathogens, encroachment in natural ecosystems, human and domestic animal population growth and the cultural mixing mainly influenced by a few centres of cultural diffusion, may have created the context for a new host-pathogen stage (Woolhouse and Gaunt 2007). How do we adapt our surveillance and control of diseases to these new contexts? Can we simply use the standard one host-one pathogen approach?

Historically, the one host-one pathogen concept has been successful in controlling and sometimes eradicating diseases of domestic and human species. In the field of animal diseases, the control of bovine tuberculosis (bTB) in Europe, and the likely global eradication of rinderpest in the entire world are commonly cited examples of successes of this standard approach (Rweyemamu and Cheneau 1995). Albeit not single host infectious diseases, the epidemiology of these two pathogens afforded the disease to be controlled by single host management. The classical approach in epidemiology consists in targeting one pathogen in one host and trying to control host-pathogen interaction by acting on the host (e.g. vaccination, treatment, culling). If past experiences such as the control of trypanosomiasis in Southern Africa was based on the perception that wildlife would play a role in disease epidemiology, only recent research has indicated that most human and domestic animal

pathogens share their epidemiological cycle with wildlife hosts (Artois et al. 2001, Cleaveland et al. 2001, Woolhouse and Gaunt 2007, Jones et al. 2008). This observation is the result of two factors: 1) the human species and its domesticated species represent only a small fraction of the biodiversity of mammals and birds. Most epidemiological cycles have more than one host (Cleaveland et al. 2001) and statistically wildlife species must represent an important fraction of the available hosts ; 2) more recently, an array of human induced worldwide changes has increased movements and contacts between humans, domestic and wild species (Daszak et al. 2000, Cleaveland et al. 2007). Movements of animals, their products (for trade purposes) and people (e.g. emigration-immigration, tourism) are used by pathogens to move with their hosts. The contacts induced by these movements, often impossible in a natural context, are used by pathogens to spill-over from one susceptible host to another. Furthermore, the encroachment of human populations into natural areas for different reasons (various socio-economical, political or demographic contexts) creates also contact between humans, domestic and wild species that would not occur naturally. As a consequence, the one host-one pathogen approach is no longer effective in many situations resulting in the need to work on complex multi-host and multi-pathogen system approaches.

Clearly, a new approach to the study of host-pathogen interactions is required. The interaction of a pathogen with one of its hosts will be modified if other susceptible hosts are present in the ecosystem. Similarly, the presence of other pathogens in the same host and in other hosts in the ecosystem will influence the outcome of the interaction between a pathogen and one host. Standard epidemiology has not taken into account this dimension of host-pathogen relation and must therefore be re-enforced by various fields of ecology, such as community ecology and evolutionary biology. Epidemiology investigates the interaction between pathogen and their hosts and therefore is a subset of ecology which looks at the interaction of organisms with their biotic and abiotic environment (Begon et al. 2006). In the

last couple of decades, integration between these fields has begun (see Kitron 1998, Galvani 2003, Guernier et al. 2004, Guégan et al. 2005, Lafferty 2009 for a few examples). Disease ecology has been proposed as a new scientific field to represent this integration (Sheldon 1997, Collinge and Ray 2006). However the full multidisciplinary integration has not been achieved mostly because of the segregation of academic careers.

This thesis aims at filling part of this gap, namely at developing the integration between the fields of epidemiology and ecology in order to address disease ecology in its new dimensions. Each scientific field has developed tools for particular purposes (e.g. molecular tools for characterising specific pathogen species, parasite community comparison for community ecology) and I have tried to use some of these tools in a combined research framework. The premise of this thesis is to shift the focus of health studies from a pathogen- or host-centred to a process-centred focus. The host-pathogen interaction is an ecological process and I defined the term of “epidemiological interaction” as any ecological interaction which results in the transmission of one or more pathogens. Looking at “epidemiological interactions” between host populations, I am interested in the ecological processes involved and try to explore the common properties of these processes. The practical goal of this thesis is to provide a new research and surveillance framework to address the risk of disease transmission (including emergence) at the human/wildlife/domestic interface.

Thesis Outline

The structure of this thesis is composed of theoretical chapters (**Chapter Two and Seven**) which aim to explain why greater integration between epidemiology and ecology is required, and explores how to theoretically and practically implement this integration. Between these theoretical chapters, four chapters develop the research framework, step by

step, using both epidemiology and ecology to explore the dynamics of avian influenza viruses at the wild/domestic bird interface in a Southern African wetland. The main chapters (1 to 8) correspond to the core of this thesis. Chapters in appendix represent complementary work achieved during the course of this thesis, for which I have been strongly involved and my contribution as a co-author reflects this. The last chapter in appendix is a requested book chapter written before main Chapter Seven and the version is different enough from this core chapter to appear in appendix.

Chapter Two (Caron et al. 2009) presents a research framework using host and pathogen data to identify host species and populations at risk of introducing, spreading and/or maintaining a specific pathogen. This chapter was developed after extensive field work on avian influenza and wild birds in Africa (**Appendix One & Two**), which continued during part of this thesis (**Appendix Four**). The epidemiology of Highly Pathogenic Avian Influenza (HPAI) H5N1 is still unclear despite the efforts of the research community. Studies bringing new insights add more variability in the host-pathogen system and uncertainty in the prediction of local risks. Global analyses of wild birds' flyways in parallel with virus outbreaks have brought limited conclusions once the raw information was extracted from relevant maps. In this chapter, we suggest an integration of epidemiology, evolutionary biology and community ecology in a research framework for a local level study (at the scale of the ecosystem). This multidisciplinary approach aims at understanding the pathogen transmission processes at the interface between different bird groups whether wild or domesticated. I believe that this ecological data brought together with the epidemiological and molecular data is a key element to explore the mechanism of the AIV ecology in their hosts.

Chapter Three (Caron et al. 2010) is a direct response to the ideas developed in **Chapter Two** on two aspects: 1) after the first snapshot done on avian influenza viruses (AIV) in Africa, we needed to explore the dynamics of AIV in some African ecosystems and

required therefore time series data in order to understand the ecology of these viruses in Africa; 2) it combines a community ecology approach on host species and a risk factor-based methodology borrowed from epidemiology to ascribe a quantitative risk to epidemiological interactions between different bird species (belonging in our case to different avian compartments as defined in the chapter). The ecology of hosts is crucial in understanding mechanisms of pathogens transmission and spread in complex multi-hosts systems. This paradigm is used to infer epidemiological interactions in the context of avian influenza virus (AIV) maintenance and spread at the interface between wild and domestic birds in an African ecosystem. I use the overlap of bird communities in space and time combined with ecological dynamic and non-dynamic risk factors to evaluate a risk of AIV introduction, maintenance and transmission between bird populations. From this, I produce hypotheses on the dynamics of circulation of AIV strains in waterfowl populations and on the potential “bridge” species at the wildlife/domestic interface. This protocol is a) reproducible and useful to explore AIV risk and identifies wild bird species potentially acting as reservoirs or spreaders of pathogens at a local scale; b) can be used as a management tool to improve surveillance at a local level. It is the first protocol to our knowledge providing a quantitative framework to identify bridge species potentially spreading AIV from wild to domestic birds and *vice versa*. I sampled the potential “bridge species” between wild and domestic bird populations identified in this chapter to test the model (on-going work).

Chapter Four (Caron et al. 2011) summarises the ecology of AIV in the wild bird community as we have observed it during two years in a Zimbabwean ecosystem. This chapter shows for the first time the persistence of AIV in a Southern African ecosystem (and in Africa as well) in waterfowl. It is an important achievement for the understanding of AIV ecology in Africa. This communication about the ecology of AIV in Africa with the suggestion that AIV are endemic in Africa is part of broader research collaborations at the

regional and continental level. **Appendix Four and Five** present these large-scale approaches (currently on-going) that I have been involved with during the course of this thesis. Waterfowl were counted and sampled in a Zimbabwean wetland over 24 months. Low Pathogenic AIV (LPAI) strains were detected during 20 consecutive months, providing the first evidence of regional yearly persistence of LPAI. I discuss the role of Afro-tropical ducks in viral maintenance and transmission and attempt to explain the observed patterns. The role of the environment is also suggested through the seasonality of rainfall and lake levels. The environment-host-pathogen link is at the core of the “One World, One Health” concept. The modification of any of these three components will have an impact on the others. One needs to take into account the full spectrum of this triptych in a particular ecosystem in order to understand which effects an action on one component will have on the others.

Chapter Five uses the data produced in the previous chapters to build a probabilistic model based on possible scenarios of spread of HPAI through wild birds from one bird compartment to another one in the study ecosystem. The question addressed is: what is the probability of spread through wild birds of a HPAI from one avian compartment to the next and can targeted control reduce significantly this risk? By using: 1) prevalence data obtained in domestic and wild bird species; 2) Community richness and abundance on host species as well as simple population dynamics model for each bird compartment; 3) Estimation of epidemiological interaction based on shared community of birds (as in **Chapter Three**), we construct a risk analysis framework to build this model. The model suggests 1) that risk varies by season; 2) limiting contact between the infected compartment and a few potential bridge species could control the majority the spread of the HPAI through wild birds; and 3) that the risk of diffusion in the entire ecosystem varies considerably with the avian compartment into which the HPAI is introduced (wild birds and ostrich farms). These findings give a direct contribution to AIV control and wild/domestic bird management in the ecosystem. If any

sanitary threat appears in one compartment, this model will point at the interactions at risk of spreading the threat from the infected compartment to another, giving the opportunity for management and control options.

Chapter Six is a comparative analysis of waterfowl communities and avian influenza ecology at a regional level using the epidemiological functional group (EFG) concept presented in **Chapter Seven**. The dataset was produced by the GAINS-SA project, consisting of the three main sites of the project, including the Zimbabwean site that I managed (**Appendix Five**). I have the possibility in this chapter to put the capture bird sample for AIV detection in perspective with the bird community present in the ecosystem. This level of study adds complexity in the analysis. However, the information extracted from the bird census data is crucial to understand the viral dynamics and detect the bias in the sampling technique. The comparison of the proportion of bird orders in the sample and in the counted community reveals which species the protocol has failed to sample and should be targeted in the future. The use of the EFG concept is helpful to reduce the complexity of bird community (with dozens of species present) by allocating bird species into groups of expected similar function in the viruses' ecology. The outputs of this chapter are threefold: 1) I can compare the dynamics of different bird community in three different ecosystems and suggest hypotheses on viral circulation based on bird ecology; 2) Applying the EFG approach, I am able to analyse the AIV results per groups of birds and validate or not my hypotheses on the function of these bird groups; 3) I conclude by challenging for Southern Africa the idea that AIV epidemiology is mainly dependant on Anseriforms and/or Charadriiforms. The remaining bird community seems to play a role in AIV epidemiology.

Chapter Seven is the second theoretical chapter. The first approach presented in detail in **Chapter Two**, using host data to infer epidemiological interactions is developed in **Chapter Three, Four, Five and Six**. In this chapter, I explore and define the basis for the

pathogen approach. Emerging infectious diseases result from the spill-over of pathogens to new species within multi-host systems. The current disease surveillance systems cannot anticipate emergences because they fail to identify future culprits (pathogens and reservoir or spreader hosts) in these complex systems. The actions of public health officers and veterinarians are restricted to later stages of epidemics once the severity of outbreaks can be much higher. However, recent advances in community ecology, molecular ecology and network analysis open new perspectives for the integration of epidemiology and ecology and for the understanding of disease transmission in multi-host systems. Shifting the focus from host-pathogen relationships to transmission processes, we develop a framework building networks of epidemiological interactions between host populations (of the same species or from different species) at the ecosystem level. These networks use two types of data: 1) Host movement and contact data (e.g. direct observation, telemetry as a proxy of disease transmission); 2) Parasite community data from different host populations, assuming that past transmission pathways inferred from this data are the most likely transmission pathways for emerging pathogens. The field of parasite community ecology has provided analytical tools to compare parasite communities by controlling for confounding factors (e.g. phylogenetic distance). We define also the concept of epidemiological functional groups to which host populations can be allocated according to their potential role in epidemiology of parasites, drawing a parallel with the approach adopted by community ecologists to assign species to functional groups. Hosts are grouped together when sharing a similar role in the transmission of a parasite or a group of parasites (e.g. reservoir, spreader, dead-end host). We explore the relevance of this approach to identify the most likely future transmission pathways between host populations in a given ecosystem. Once identified, these transmission pathways can be targeted by disease surveillance and control to prevent the next pathogen emergence. The epidemiological interaction network framework that we present could achieve two objectives:

increasing theoretical knowledge on the ecology of disease transmission and on multi-host multi-pathogen interactions and providing a tool for EID early detection. In **Appendix Seven**, I have a slightly different angle of approach to define the same ideas and put more emphasis on some key aspects or potential weakness of the methodology (this Appendix is an invited book chapter).

In **Chapter Eight**, I summarise the main findings of my thesis and how they contribute to disease ecology and, more precisely, to transmission ecology. Transmission ecology focuses on the process of transmission instead of focusing on one host or one pathogen. I try to discuss how (or to what extent) the transmission mechanisms in a particular ecosystem are largely independent of particular parasite species. The common properties of transmission processes enable the development of new hypotheses that can be tested to better understand host-pathogen interactions in a complex system, particularly at the wildlife/livestock interface



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Chapter Two: Evolutionary Biology, Community Ecology and Avian Influenza Research

(Chapter reference: Caron, A., Gaidet N., de Garine-Wichatitsky, M., Morand, S., Cameron, E. (2009) Evolutionary Biology, Community Ecology and Avian Influenza Research.

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Background and context

The emergence in 1997 of the HPAI H5N1 strain (Highly Pathogenic Avian Influenza, Asian lineage) followed by its rapid spread and evolution across three continents challenges the previous understanding of host ecology and evolutionary processes of Avian Influenza Viruses (AIV). Before this emergence, the current understanding of the virus ecology in its hosts was that LPAI (Low Pathogenic) strains circulating in wild birds were acquiring their HP properties once introduced into gallinaceous domestic poultry without spill over to wild birds (with rare exceptions) (Alexander 2000, Webster et al. 2007). Therefore the interaction of AIV between wild and domestic birds was considered unidirectional. One of the specificities of the HPAI H5N1 strains is its potential to infect a wide range of both wild and domestic bird species (Webster et al. 2007). The coexistence of this HP strain in these two groups of hosts provides the potential for bilateral interactions between domestic and wild birds for the maintenance and evolution of the pathogen.

Avian systems, which can be classified broadly as “natural” versus “domestic” systems, have been studied through two radically different approaches by distinct bodies of academics (e.g. veterinarians and ornithologists). The integration of these fields of study in a common framework research should benefit the understanding of the HPAI H5N1 ecology and epidemiology. These multiple host – multiple pathogen interactions are complex and difficult to tackle within classical veterinary science, and increasingly multidisciplinary research flourishes.

We here use the theory of evolutionary biology and community ecology to investigate the processes of emergence, maintenance and evolution of AIV with HP characteristics in the avian hosts. Evolutionary biology aims at describing the interaction of organisms through the underlying mechanism of natural selection (Morin 1999). Natural selection can explain why,

for example, microorganisms such as viruses, with a short life cycle, evolve rapidly and pose a threat to other organisms with longer life cycles such as vertebrates. A community is an assemblage of species populations that coexist spatially and temporally and potentially interact (Begon et al. 2006). The processes of viral evolution, with genes as the unit of selection, are dependent on the structure of the host and pathogen communities: if different selection pressures occur in different avian populations, virus strains spreading from one population to another will experience a different evolutionary pathway. As community structures are dependent on biotic and abiotic factors, virus evolution will vary between ecosystems and regions.

An organism with its genes is the product of past adaptations to its environment (evolution) and the on-going interactions with the present biotic and abiotic surroundings (ecology). This dynamic view of an epidemiological case is essential to describe, predict and control the HPAI H5N1 epizooty.

Current understanding of AIV Epidemiology

Two major types of AIV are distinguished by their pathogenicity. They are classified as LPAI and HPAI viruses based on either their clinical lethality (measured on experimentally infected 10-day old chickens) or on molecular characteristics (sequencing of the hemagglutinin cleavage site) (OIE 2005). It is increasingly recognised that these definitions do not completely overlap. The clinical definition of pathogenicity is determined for domestic poultry only (Lee et al. 2007). A vast majority of AIV with high lethality in gallinaceous poultry fit the molecular definition; however the acquisition of high pathogenicity is recognised to be multigenic and cannot rely only on the HA molecular characteristic. The major transmission route for AIV strains is through faecal–oral transmission.

Wild waterbirds, particularly species of Anseriformes (ducks, swans and geese) and Charadriiformes (gulls, terns and waders), are the natural hosts for LPAI strains with endemicity and seasonal high prevalence in some species (Alexander 2000, Olsen et al. 2006). Data is not available from all waterbirds populations, but given worldwide surveillance, they seem to play the role of an AI strain reservoir and host a viral genetic pool in complex evolution but with low pathogenic impact (Chen and Holmes 2006, Webster et al. 2007). In gallinaceous poultry, LPAI strains appear to be occasionally found and do not seem to be maintained though this could have been overlooked (Alexander 2007).

HPAI strains were detected mainly in gallinaceous poultry and occasionally in terrestrial birds around these outbreaks. In waterbirds, no HPAI have been found with few exceptions: a H5N3 lethal in terns (Becker 1966) and a H5N2 with a HP genotype in healthy wild ducks (Gaidet et al. 2008). Currently, only H5 and H7 (and to a lesser extent H9) subtypes have been found to be highly pathogenic. These HPAI found in poultry outbreaks have been phylogenetically linked to LPAI maintained in wild waterbirds reservoir (Munster et al. 2005, Campitelli et al. 2008). The understanding is that the available viral gene pool in waterfowl populations feeds the gene pool in domestic poultry (Alexander and Brown 2000).

HPAI H5N1 epidemiology

HPAI H5N1 epidemiological patterns do not conform to those of other HPAI. It is renowned for: a) a broader host range, with new bird and mammal species infected: particularly, domestic ducks and a vast range of wild birds (Chen et al. 2005, Webster et al. 2006); b) its heterogeneous pathogenicity for wild (Brown et al. 2006, Keawcharoen et al. 2008) and domestic (Sturm-Ramirez et al. 2005) waterfowl ; c) its increasing environmental

stability (Brown et al. 2007, Lowen et al. 2007, Park and Glass 2007); d) tracheal excretion more important than faecal excretion (Sturm-Ramirez et al. 2005, Antarasena et al. 2006).

The striking difference in the epidemiology of HPAI H5N1 is the co-occurrence of this pathogen in both wild and domestic birds. Dead wild birds have been found infected with the virus in Asia (Chen et al. 2004), Europe (Komar and Olsen 2008) and Africa (Ducatez et al. 2007a). Experimental inoculation studies of HPAI H5N1 have resulted in susceptibility in all bird species tested: the response is highly variable at the inter- and intra-specific level, with some individuals developing symptoms and dying while others remain asymptomatic (Brown et al. 2006, Pantin-Jackwood et al. 2007, Pasick et al. 2007, Keawcharoen et al. 2008). However, no HPAI H5N1 have been detected in waterbirds despite a massive international surveillance effort in recent years (Appendix One - Gaidet et al. 2007, Krauss et al. 2007, Wallensten et al. 2007) apart from few reported cases (Kou et al. 2005, Chen et al. 2006, FAO 2008a). This results needs to be interpreted according to the millions of waterbirds estimated but suggest that HPAI H5N1 is not circulating intensively in wild birds.

The intensive poultry production unit could play the role of reservoir despite the high mortality experienced: a metapopulation model can explain this phenomenon. Waves of infection leave behind recovered (resistant), or dead, individuals and re-infection occurs when the virus is introduced into a naïve population (new or re-stocked). However, the field data from South-East Asia do not support this hypothesis: despite large stamping-out and additional control measures, the HPAI H5N1 seems to be endemic (Chen et al. 2004, Hulse-Post et al. 2005, Sturm-Ramirez et al. 2005, Gilbert et al. 2006a). The farming of free-ranging domestic ducks in paddy fields provides an epidemiological key explaining the making of an artificial reservoir (Songserm et al. 2006, Buranathai et al. 2007) although the mechanisms are unclear. However, this type of farming is not present in Europe and far less extensive in Africa where the virus nevertheless persists. In Europe, it is not known if the virus has

become endemic in an avian population. Reoccurrence of outbreaks with spatial spread including mortality in sentinel species such as swans raise the hypotheses of a reservoir to be discovered.

In summary, it seems unlikely that either wild birds or the poultry production units independently act as reservoir hosts. Therefore the community interactions between compartments need to be investigated as a mechanism by which the disease could be maintained. Considering the common origin of AIV strains and the exchanges of strains between wild and domestic birds, what makes a particular virus becoming HP or LP? Differences in host ecology need to be delineated to start understanding variables at stake.

Avian host ecology

Knowledge on population dynamics of waterfowl communities is based on decades of intensive ornithological studies. This data has provided support for research investigating the possible roles of wild birds in the spread of AIV, but is still lacking sufficient precision - the measurement of movement parameters - to answer epidemiological questions (Olsen et al., 2006). Wild waterfowl represent a large part of avian biodiversity and a broad genetic heterogeneity compared to the few domesticated species. They have relatively long life cycles and live under variable densities, presumably with multiple exposures to pathogens and a well-developed immunity. These hosts share an evolutionary history with their pathogens. Some waterbirds are also characterised by their gregariousness, including interspecific mixing in different environments, and they have different movement patterns (nomadic, resident or migratory).

Conversely, the domestic poultry production system is characterised by low diversity and high productivity in artificial systems, particularly in the last few decades. In 2006, gallinaceous poultry (92.5% chicken, 1.2% turkey) represented the bulk of domestic production with ducks only reaching 5% (FAO, 2008b). Over 50 billions of poultry heads produced in 2006 presented in this database are to be compared to the millions of waterfowls estimated. Intensive poultry are raised under very high densities (more than 20 birds per square meters for broilers) and for short life cycle rarely exceeding 36 days (for broilers) with very little chance of immunity development (enhanced by antibiotics use). Most birds in poultry population are not breeders and do not participate to the next batch. In addition, the current globalisation of the poultry trade tends to homogenise the breeds used resulting in homogeneity of existing genomes and day-old chicks globally available from any origin.

In between these two domestic and wild systems, a significant proportion of avian populations is raised under a mix of these life history traits, such as backyard chickens production and farmed ostriches. How can we associate the current knowledge in the virus epidemiology with this variability in host ecology?

Role of interactions of hosts between compartments in AIV evolution

These wild and domestic populations can be clustered into a network of “compartments” (Figure 2.1) each one defined as a set of avian populations under similar environmental drivers: chicken in an intensive production building would be part of the intensive poultry production compartment for example; whereas waterfowl form another compartment. The key to understanding AIV epidemiology and particularly the current HPAI H5N1 threat may be in the interaction between compartments, and the different evolutionary processes in these systems. Interactions between these compartments can occur through direct

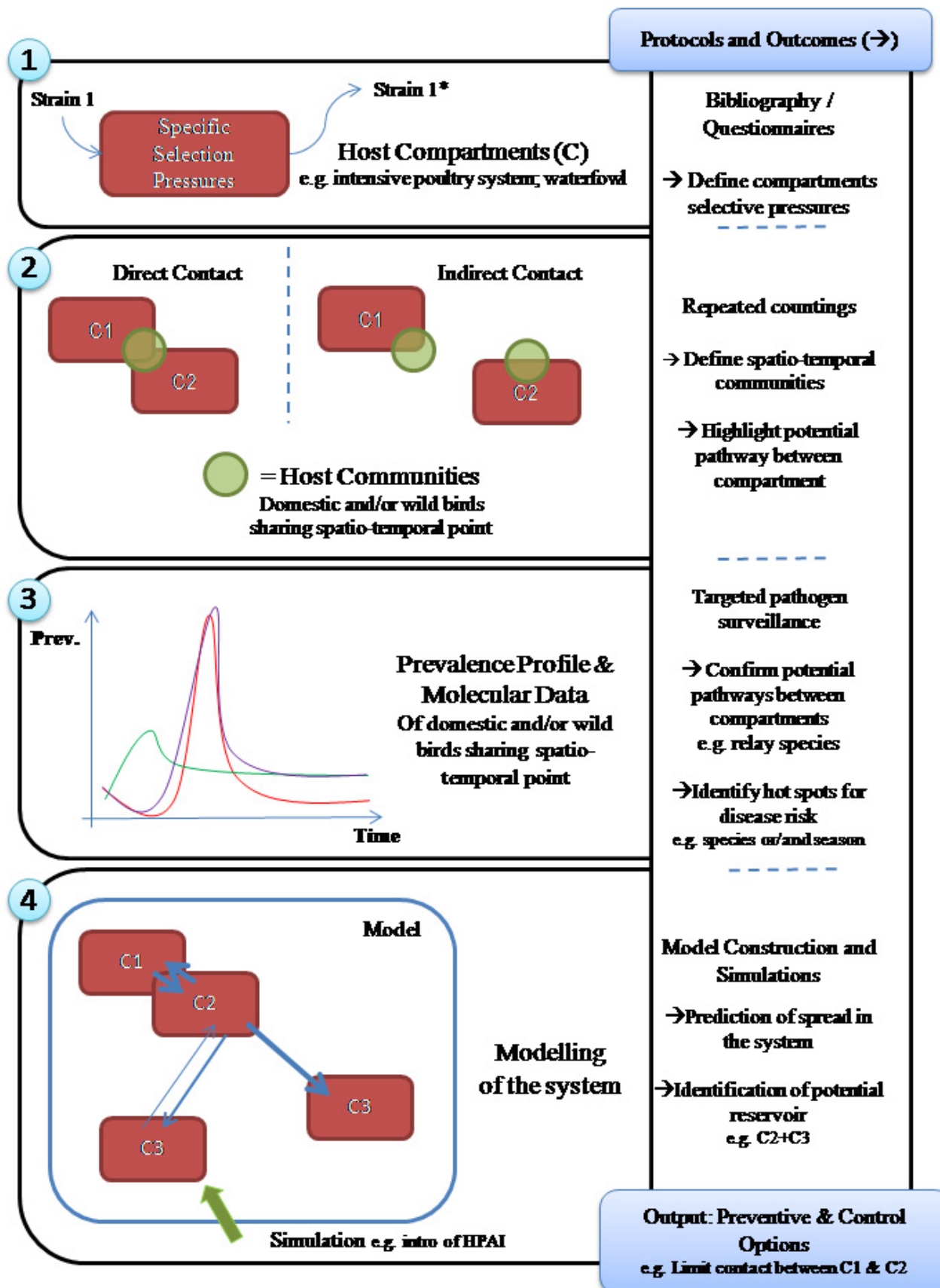
contacts– i.e. mixing of waterfowls and domestic birds - or management practices – i.e. free-ranging farming -. Indirect contacts, poorly studied yet, can also occur when wild “bridge” species share space and/or time with birds in two different compartments.

These interactions will draw potential pathways for viruses spread between compartments. As compartments are defined by a set of selective pressures, can one predict viral evolution in their specific context?

The main evolutionary mechanisms for AIV are high mutation frequency, reassortment (both leading to antigenic drift) and recombination (Webster and Hulse 2004), making them rapidly evolving organisms (Boyce et al. 2008). The high rate of co-infection, notably in waterfowls (Sharp et al. 1997), enhances reassortment (Wang et al. 2008). The different avian compartments will drive viral evolution through these mechanisms, but the different environments will have different evolutionary consequences.



Figure 2.1: *Conceptual research framework using community ecology and evolutionary biology to explore AIV ecology within their host in ecosystems. Four steps are presented referring to numbers on top left of each box: 1) Definition of domestic and wild compartments in the ecosystem; 2) Definition of the communities (Begon et al., 2006) in the ecosystems (temporal and spatial variation); 3) Targeted surveillance to test hypotheses from (2); strains isolation will confirm or not prediction on host selective pressure on viral evolution per compartment; 4) Modelling of the system for predictive purpose and definition of new hypotheses – feedback to (2) and/or (3). On the right vertical box, protocols and outcomes (preceded by arrow) proposed for each box. As an example we could consider: C1= backyard poultry compartment; C2=waterfowl compartment; C3= Intensive poultry compartment.*



Intensive poultry production units are an ideal viral breeding ground due to: a) Genetic homogeneity, decreasing the number and variability of resistance genes, little variation between individuals and therefore high infection potential for viruses; b) No reproduction for the majority of birds prevent any co-evolution with the pathogen providing the conditions for a highly lethal strain to be maintained; c) Artificial density of individual hosts increases spread to other individuals; d) Short life cycles prevent the host organism from developing immunity; e) In a compartment with high density and short life spans contagiousness is not under selective pressure and a selection criteria on strains, allowing strains to be selected mainly on virulence (harm to the host; linked to host exploitation; Read and Taylor 2001) ; f) Sometimes inadequate disinfection measures between batches allow the virus to survive in the environment. These suggest some direct inferences: the change in excretion of the H5N1 HPAI could be an adaptation of the virus to intensive production systems where infection would be enhanced by aerosol transmission, whereby a three dimensional space can be utilized for spread compared to faecal transmission which is constrained in a two dimensional space on the ground.

This combination of variables will select for viruses with high virulence, as other life history traits are released from selective pressure (Read and Harvey 1993). Therefore, a highly virulent mutation which would be selected against under natural conditions (since spread would be limited at low density) will thrive in these conditions. In intensive poultry production systems, HPAI strains, if introduced or created will be selected. HPAI H5N1 could be a product of such selective process.

One critical research area for the ecology of HPAI H5N1 in avian populations is interactions, both intra- and inter-strain, with other pathogens and with the host's immune system. Data on the variability of HPAI H5N1 strains is available due to the increased surveillance targeting this specific strain (de Jong and Hien 2006, Ducatez et al. 2007b,

Salzberg et al. 2007, Starick et al. 2007, Zohari et al. 2008) and new HPAI strains are identified (Monne et al. 2008). Since 2004, different strain types from the same clade have circulated in South-East Asia before spreading to other continents. This data could fit in the model of strains with increased virulence which would outcompete previous dominant strain types. As these strains types spread to other domestic or wild avian populations, they would compete with other LPAI strains. The result of intra-specific competition would be dependent on their environment, particularly the host population. The outcome could be co-infection of avirulent pathogens or the selection of a highly virulent pathogen (Van Baalen 1998): 1) the LPAI-waterfowl system illustrates the first alternative. Longitudinal studies in North America (Krauss et al. 2004) and Europe (Munster et al. 2007) have described two year cycles of LPAI strains in wild bird populations, indicating competition between strains, an effect of acquired host immunity and turnover in a population of susceptible host through recruitment; 2) the second alternative is represented by HPAI strains selected in intensive domestic populations with an environment enabling them to outcompete other less virulent strains. Consequently, the penetration of HPAI H5N1 strains (selected for their high virulence in intensive poultry) in the co-evolved systems between waterfowl and LPAI would be difficult: low density of host population leading to a selection on contagiousness, a developed host immunity inducing resistance and the presence of intra-strain competition with other AIV will all be factors against their maintenance and spread. The hypothesis that HPAI H5N1 could persist in some wild populations is not supported by this theoretical approach except through a meta-population mechanism with host population extinction and spread dynamics. The susceptibility of each wild species to HP strains is the missing variable to test this model. Finally, competition between different pathogens at both the population and individual levels are under-studied. For example, Newcastle disease is widespread and induces more than 50% of mortality in domestic avian populations infected. The role of wild birds in the

epidemiology is unclear. A HPAI strain infecting a population where Newcastle disease occurs will be in a direct competition for hosts. The outcome of this competition is difficult to predict but will be important for the maintenance of HPAI strains in wild populations.

Gene reassortment can occur when two strains co-infect one host individual. It combines the genetic history of two strains which have evolved in different environments. This could result in an increase of host range (Webster et al. 2006) and pandemic threats (Nelson et al. 2008). Co-infection is therefore important, and dependent on the evolutionary interactions between different strains (intraspecific competition) and the host immunity. Reassorting strains selected in different environments will create more variable new subtypes than strains selected under similar selection pressures. Reassortment between LPAI should occur often in wild bird populations with a low probability of making of a HPAI. However, HPAI, selected in intensive poultry units, mixing with a LPAI (event with a low probability) can acquire more variable life history traits, leading to hazardous mixing for animal and public health. However, these events will depend on the degree of interaction between domestic and wild bird populations. For example, the gene pool from the American waterfowl community is separated from the one of Eurasian origin with limited exchanges despite overlap in wild birds migrating flyways (Olsen et al. 2006; Krauss et al., 2007); practices such as the farming of domestic ducks, the raising of mallard ducks for hunting in Europe and the extensive farming of ostriches in Southern Africa artificially places a species in potential contact with different compartments under different selection pressures and hazardous mixing possibilities

Implication of evolutionary biology and community ecology for AIV research

The epidemiological dynamics of HPAI H5N1 in different regions seem to be variable. The global analysis of bird migrations has provided until now little information in understanding the pattern of geographical spread (Gilbert et al. 2006b): the lack of information for a large number of species, variation between individuals (age and gender) and populations impede the generalisation from the data used. Each region at risk will differ in species composition, spatio-temporal patterns of movement and contact between domestic and wild avian populations. Compartment specificities will drive strain evolution toward different pathways creating “selection mosaics” or a “geographical evolution of virulence” (Thompson 1999, Hochberg et al. 2000). Similarly, phylogenetic analysis is an important tool but has limitations: with the detection of related strains at two distinct spatio-temporal points one can only speculate on virus spread or origin. Recent studies have suggested that LPAI strains can influence migration success of a swan species (van Gils et al. 2007). If confirmed and generalised to other strains and hosts, there could be a negative relationship between strain virulence and the distance travelled. Furthermore, if birds infected with LPAI migrate less efficiently, the bird populations at departure will decrease in size but with a higher concentration of infected birds. The average migration departure time is not representative of the movement of infected birds and the research should be concentrated on the tail of this migration distribution profile.

Therefore we suggest that a local approach (Figure 2.1) may enhance our understanding of the virus epidemiology, using community ecology to evaluate contact patterns between compartments. Such an approach will provide a cross-cutting analysis of the compartment-specific selection pressures on viruses and the intra-community interactions that

define the potential spread pathway. For example, a rural village close to a wetland will host two avian compartments: a backyard flock and a waterfowl population. Regular bird counts at particular places will identify any direct or indirect contact between compartments. Targeted disease surveillance, relevant for each bird compartment, should be used to test hypotheses on the maintenance and spread of the virus using molecular techniques. A weakness of this approach lies in the low prevalence and high sample size necessary for AIV isolation and the financial cost for using additional efficient molecular techniques. Taking into account the community of hosts will be important if the reservoir of LPAI or HPAI is not a single species but a complex interaction of bird populations (wild and/or domestic) in a specific spatio-temporal range. The concept of a multi-host reservoir (Haydon et al. 2002) could explain the maintenance of HPAI H5N1 in some ecosystems.

From this perspective, specific practices could be targeted to implement a compartment/community study. Domestic ducks in South-East Asia seem to act as a reservoir for HPAI H5N1. This is an interesting compartment since this domestic species is free-ranging during the day, in contact with the waterfowl compartment, and in farm buildings at high density at night. The potential for virus acquisition and selection is therefore high, and this interface compartment seems at risk. Mallard ducks raised for hunting in Europe may also be a potential “Trojan Horse” (Webster et al. 2007), since this wild species is kept under a domestic poultry management scheme for future release in the wild, with obvious differential selective pressures, and it plays an important role in LPAI epidemiology. The extensive farming of ostriches in Southern Africa is also of concern with recent HPAI H5N2 outbreaks: this wild but recently domesticated species, free-ranging and potentially in contact with wild populations has been highlighted as a potential mixing vessel for AIV (Abolnik et al. 2007). These practices promote contacts between domestic and wild bird populations and the created

interfaces associate the gene pool and the gene selection process which should lead to selection of HPAI strains.

Conclusion: describing the pathogen risk

HPAI H5N1 introduction pathways are a nightmare for epidemiologists (see recent introduction in UK or France as examples). The variability in individual bird migration coupled with intra-specific variability of susceptibility to HPAI H5N1 has been demonstrated by recent studies. More variability lies in the AIV interactions with their environment: other AIV strains, other pathogens, and the host immune system. Future researches have no options other than being local and specific.

We advocate a community ecology approach using evolutionary theory and phylogenetic analysis to build and test between-compartment interaction hypotheses. This ecological and epidemiological data can then be integrated into a risk analysis model (Goutard et al. 2007). Once compartment interactions are understood, the consequences of a virtual viral introduction in one of the compartments spreading to other compartments can be modelled and its risk estimated. Seasonally, pathways for virus spread in the system could be identified by simulations. The linkage of multiple local analyses could lead to a global understanding of the eco-epidemiology of the virus. Real and Childs (2006) have presented a similar approach for rabies studies at a global scale.

The community/compartment approach could be extended to human communities with the help of social sciences. Fasina et al. (2007) explains how peri-urban human populations in Africa are at higher risk of HPAI H5N1 infection than others. Such a human compartment has

specific interactions with the backyard poultry compartment and the wild bird compartment.
This type of model could help describing the pandemic risk.



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Chapter Three: Estimating dynamic risk factors for pathogen transmission using community-level bird census data at the wildlife/domestic interface

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Introduction

The success of multi-host infectious pathogens in ecosystems is heavily dependent on the composition of the community of organisms in which they occur (Ostfeld 2009). The species composition of the host community and the temporal dynamics of its constituent populations will influence pathogen success through variation in such parameters as host susceptibility, host abundance, host population turn-over, the presence and absence of reservoir species, and encounter rates between hosts and pathogens (Dwyer et al. 1997, Childs et al. 2007, Borer et al. 2009).

For pathogens that are transmissible either by direct contact or via the shared use of the same habitat at different times, transmission parameters often cannot be directly measured in the field. Doing so is particularly difficult for multi-host pathogens. Transmission is usually evaluated through host-pathogen models (Breban et al. 2009, Rohani et al. 2009) that lack direct measurements of actual interspecies contact. Epidemiological interactions (i.e., ecological interactions that may result in the transmission of a pathogen) between susceptible, infected, and recovered hosts can be used to define a network from which to explore transmission pathways and assess spatial and temporal variation in transmission risks (Takeuchi and Yamamoto 2006, Duerr et al. 2007, Kenah and Robins 2007). While graph theoretic methods for creating and analyzing networks from direct data on species interactions are fairly well established (Williams et al. 2002, Lafferty et al. 2008), the application of standard network methods in cases where interactions and mechanisms must be inferred from higher-level data on co-occurrences is poorly developed and computationally challenging (Bascompte and Melian 2005, Rabbat et al. 2008).

Here we consider the use of co-occurrence data to infer avian influenza virus (AIV) potential transmission pathways in communities of birds in Zimbabwe. The recent HPAI

H5N1 (Highly Pathogenic AI) panzootic has spread across the world, exploiting avian communities and sporadically infecting humans (Webster et al. 2007). The mechanisms of AI spread across ecosystems are still unclear. The international poultry trade and waterfowl migration are the two most intensively tested hypotheses that have been proposed to explain patterns of HPAI spread (Olsen et al. 2006). However, existing information implies different roles for different modes of dispersal across regions, indicating a need for regional or infra-regional research frameworks (Kilpatrick et al. 2006). The epidemiology of LPAI (Low Pathogenic AI) is better understood than that of HPAI; waterfowl are considered to be the primary reservoirs of LPAI with spill-over to domestic poultry occurring periodically (Webster et al. 1992). These cross-species transmission events can lead to HPAI selection in domestic populations (Chapter Two - Caron et al. 2009). We use AIV as a complex multi-host pathogen model with a potentially high impact on the socio-economic level for Africa and the world.

The importance of the ecology of wild birds in the epidemiology of AIV strains has been underlined by numerous studies (Olsen et al. 2006, Stallknecht and Brown 2007, McCallum et al. 2008, Munster and Fouchier 2009), but the high diversity of potential host species and a lack of information on their susceptibilities to LPAI and HPAI makes the overall picture unclear (Perkins and Swayne 2002, 2003, Brown et al. 2006, Brown et al. 2007a, Pasick et al. 2007). Some key features of waterbird ecology are thought to strongly facilitate virus maintenance or spread. These features include: their relatively high degree of inter- and intra-specific mixing; their tendency to move long distances during annual migrations and/or broad-scale nomadic movements; their colonial feeding and roosting habits; and their use of water, which improves viral survival outside the host. Some studies have already used these criteria to estimate hotspots of potential virus infection, regional spread, or inter-continental contamination (Kilpatrick et al. 2006, Veen et al. 2007, Cumming et al.

2008). However, at a local level, most AI risk factors show seasonal variation as species breed and as they respond to variations in resource availability, rainfall, the presence or absence of other species (including pathogens), and seasonal changes in human behavior. The corresponding variation in AI risk has not been thoroughly analyzed in wild bird communities.

In addition to the many uncertainties regarding spatiotemporal variation in transmission pathways, it is worth noting that most current field research still follows traditional distinctions: veterinarians investigate the health of domestic species and ornithologists focus on wild birds, but the gap between these two approaches is poorly filled.

The classical one-pathogen approach aims at detecting (directly or not) the pathogen in different hosts and inferring transmission pathways that are specific to this pathogen (Plowright et al. 2008). In this article we present a novel approach to assessing transmission risks in a complex epidemiological network that consists of spatiotemporally variable bird communities (i.e., waterbirds, domestic birds, and bridge species that interact with both wild and domestic communities). Rather than attempting to develop a formal network-based model, we integrate data on the frequency and intensity of inter- and intraspecific co-occurrences, together with information about relevant aspects of species ecology and behavior, to obtain a risk score for each species in the community and to build an adapted risk assessment model. In conceptual terms, this approach offers a mid-point between data-intensive, mechanistic network analysis (Takeuchi and Yamamoto 2006) and looser, more subjective assessments of risk (Cumming et al. 2008, Peterson and Williams 2008). Our approach has the advantage that it incorporates aspects of fine-scale transmission mechanisms while not being excessively data-demanding; the analysis is undertaken using the kinds of survey data that standard ornithological censusing procedures typically yield. In addition to presenting a useful picture of seasonal variation in AI risk, our analysis demonstrates how

dynamic aspects of risk can still be included into epidemiological risk assessment in the absence of detailed pair-by-pair interaction data.

Methods

Study site

We undertook this study in the Manyame catchment (E30°30'30'', S17°45'45''), located 35 km West of Harare, the capital city of Zimbabwe. Our primary study sites were two impoundments, Lake Chivero and Lake Manyame, both of which were created in 1952. Together they form a linked wetland system (connected by the Manyame River) that harbors a community of waterfowl species. Part of the shoreline of Lake Chivero is a protected area. In addition, several commercial farms are located in the Manyame catchment, including industrial poultry farms and semi-extensive ostrich farms. Farm employees living in compounds located on the farm estates also raise backyard chickens for domestic consumption.

We considered the different avian communities in the study area to be four 'compartments' as defined in Chapter Two - Caron et al. 2009: (1) the waterfowl compartment, consisting of the community of wild waterbird species sharing the lake habitat through the year; (2) the industrial compartment, being the population of domestic chickens raised in buildings at high densities for a period of about 40 days; (3) the backyard chicken compartment, in which chicken populations are free-ranging during the day, using fields and human-modified natural habitats in the vicinity of compounds, and resting in chicken pens at night; and (4) the ostrich farm compartment, consisting of a few hundred birds kept in open paddocks (usually around a hundred birds per paddock) surrounded by wooden fences. These

different management practices result in variable contacts between domestic poultry and the surrounding wild bird communities, and biosecurity measures are implemented in intensive poultry and ostrich farms.

It is important to note that the status of AIV in this ecosystem is unknown. No H5N1 outbreaks have been recorded south of the equator in Africa. Outbreaks of H5N2 in the southern part of Zimbabwe in ostrich farms occurred in 2005, which had a possible link with outbreaks of the same strain in South Africa in 2004 (Abolnik et al. 2006, Sinclair et al. 2005).

The methodology followed a six-step process: (1) identify the hazard in relation to the objective; (2) describe the waterfowl and domestic bird communities; (3) define dynamic and non-dynamic ecological risk factors (RFs) for the presence of AIV infection in the waterfowl community; (4) combine RFs for both the release assessment (introduction risk - IR) and the exposure assessment (maintenance risk - MR) in the waterfowl community; (5) identify epidemiological interactions between waterfowl and domestic compartments through direct contact, indirect contact via shared habitat or potential bridge species; and (6) estimate the release assessment for each of the domestic compartments (transmission from waterfowl to the domestic compartments) through a dynamic Domestic Risk variable (DR).

Hazard identification

The risk of AIV introduction from the waterfowl community to the three domestic compartments is dependent on the introduction of strains in the waterfowl community, the ability of this community to maintain such strains and the potential for spill-over from the waterfowl compartment to the domestic compartments. This risk increases from non-H5 and H7 LPAI (which can still recombine with other strains to produce HP strains) to H5 and H7

LPAI (which are the most likely strains to evolve into HPAI) to already high pathogenicity (HP) strains (including HPAI H5N1). Because of the little epidemiological information available for African bird species and because any AIV strain could be involved in the creation of HP strains, we identified all AIV strains as hazardous for this risk assessment.

Community composition

Focal counts were undertaken to estimate species diversity and the abundance of waterfowl and domestic communities. Based on local knowledge of the field site, 15 shoreline sites were selected for their high diversity of waterfowl species and abundance of birds. From May 2007 to March 2009, bird community counts were carried out every two months at each of these sites. Four 30-minute counts, each at a different time of the day (06:00-09:00; 09:00-12:00; 12:00-15:00; and 15:00-18:00) were carried out in a random sequence at each site for each recording session. Prior to each count, the counter waited for 10 minutes to habituate the birds to the presence of the counter. During each count, the counter stood or sat at a distance of 30-50 m from the lake shore and recorded all birds in a 150-m-radius semicircle.

In a radius of ten kilometers from the lake shoreline, we selected 19 domestic compartment sites, located in (or in direct proximity to) production units (buildings, paddocks, or villages). Six sites in three different ostrich farms, seven sites in intensive poultry farms and six sites in villages with backyard poultry were selected. At each of these domestic sites, the same counting protocol (10-minute wait plus 30-minute count) was applied from June 2008 to April 2009 with both wild and domestic birds being counted.

Ecological Risk Factors (RFs)

The use of variables that capture ecologically relevant variation to build epidemiological RFs has been applied in different studies related to AIV maintenance and spread at regional or continental scales (Kilpatrick et al. 2006, Veen et al. 2007, Cumming et al. 2008). We were interested in describing, at the community level, the risk of introduction and maintenance of AIV within and between bird communities across seasons in response to variability in host ecology. We thus developed dynamic RFs based on seven ecological variables that were likely to influence the epidemiology of AIV, including two variables for the introduction risk (estimated local immigration and risk related to AIV strain in relation to the origin of the birds) and five variables for the maintenance risk (the overall abundance of birds, the gregariousness of the species, interspecific aggregation, percentage of juveniles and feeding habits) (Stallknecht et al. 1990b, Olsen et al. 2006). These risk factors were characterized as RFs 1 to 7 (Table 3.1). Susceptibility to AIV infection and the immunological status of the birds were not considered in this model because of a lack of information for African bird species.



Table 3.1: Risk factors (RFs) used in this study, their derivation, and the motivation for including them.

Risk	RF	Name	Properties	Description
<i>Introduction</i>	1	<i>Immigration</i>	Dynamic	Any bird arriving in the ecosystem can potentially carry a strain of influenza from another ecosystem. We quantified immigration conservatively, as the difference between the number of birds observed in a count at time t and a count in the same location at time $t-1$. Negative changes (emigration) were entered as zeros. No value for this RF exists for the first count session by definition.
	2	<i>Related AIV Risk</i>	Non-dynamic	Birds can introduce different pathogens from different areas. The local risk (notably for farmers) is therefore related to the type of strains that are likely to be introduced. We defined four movement patterns and ranked them according to the associated risk of introducing different strains of AIV in Chivero-Manyame ecosystem: a) resident species, associated with risk value of 0; b) species nomadic in Southern Africa, associated with a risk value of 1 (HPAI H5N1 has not been recorded in African South of the equator -OIE 2009- but other HP strains have been recorded) or 0 for H5N1 risk; c) Trans-equatorial migrants, with a risk value of 2 as HPAI H5N1 is now endemic in some African countries and outbreaks occurred in 11 countries (OIE 2009); and d) palearctic migrants, associated with a risk value of 3 because of the high number of HPAI H5N1 outbreaks and reported prevalence of LPAI is higher than in Africa (Olsen et al. 2006). For species that evidence several different strategies, as with the wood sandpiper <i>Tringa glareola</i> which has both migratory and resident populations (Underhill et al. 1999, Hockey et al. 2005), a mean between the two relevant coefficients was taken.
<i>Maintenance</i>	3	<i>Abundance</i>	Dynamic	Total number of bird observed per species, obtained by summing numbers seen during the 60 counts. Note that since only 56 counts were done during the first count session (May 2007), we multiplied the numbers of birds recorded during this session by 60/56 for full comparability.
	4	<i>Gregariousness</i>	Dynamic	The degree of intra-species aggregation. Aggregation facilitates pathogen transmission and maintenance in the species. For each species we calculated the average group size observed across all study sites.
	5	<i>Mixing</i>	Dynamic	The degree of inter-specific aggregation, which facilitates pathogen transmission from one species to another. We estimated the degree of mixing for each species and for each count session as the ratio of the

				number of species observed on the same sites and at the same time, divided by the total number of species counted during the 60 counts of the count session (total species diversity measured during a count session).
	6	<i>Percentage of juveniles in the population</i>	Dynamic	Juveniles are considered to play a role in the epidemiology of AIV once they have joined the adult population (i.e., after fledging). Juveniles are also thought to remain epidemiologically naïve in the population for about 2 months (Stallknecht et al. 1990b). To capture this risk, we used Roberts' Birds of Southern Africa (Hockey et al. 2005) to provide data on: a) clutch size; b) breeding success; and c) laying dates for the 254 species in the data set. Using a simple population model assuming constant mortality in adults (4,5% per month) and a decreasing mortality in juveniles (starting at 40% in month 1 and reaching 4,5% at 6 months), and integrating the reproductive information, the percentage of juveniles in the population was estimated by month. Incubation and fledging periods were added to determine the delay between egg laying and the entry of juveniles into the population. We considered juveniles for each species to be susceptible to AIV infection based on their naïve immunological status but despite lack of information on susceptibility for most African species.
	7	<i>Feeding habits</i>	Non-dynamic	Transmission of AIV strains in surface water is possible (Stallknecht et al. 1990a, Brown et al. 2007b), and we identified four feeding behaviors that were ranked according to the risk of birds being infected with AI during their feeding activities. They include: (0) feeding on insects on flight, seeds, nectar or fruits; (1) feeding on birds, small vertebrates, or insects close to water; (2) diving or feeding on insects gleaned from open water; and (3) dabbling, gleaning on or near surface and subsurface vegetation, or probing.

Introduction and maintenance risk (IR and MR) in waterfowl community

For each bird community count, the species values of each RF were multiplied by 1 for species recorded at least once and by 0 when the species was absent. IR and MR were calculated as described in Table 3.2. IR was calculated for any AIV strain and specifically for HPAI H5N1 in order to display the proportion of the relative risk for exposing the community to HPAI H5N1 introduction.

The standard deviation of each RF of the MR was calculated. A Spearman Rank Correlation test was performed for each RF in relation to the MR in order to assess their relative contributions.

Quantifying epidemiological interactions (domestic risk - DR) and their risks

Calculating the degree of ecological interaction between wild and domestic compartments

Each waterfowl count session was paired with count sessions carried out in domestic compartments (separated by a maximum of three weeks). The community composition of each domestic compartment was calculated using the same method as for the waterfowl compartment. For each domestic compartment, all species seen during the same session in the waterfowl compartment were identified as the shared community. We calculated the proportion of the shared community for each domestic compartment potentially in contact with the waterfowl compartment during the same period.

Calculating the interaction risk (DR) of the shared community

For each species recorded in the waterfowl and domestic compartments during the same period, the DR was calculated as described in Table 3.2. For each session, we estimated

the DR (i.e. of AIV spreading from the waterfowl to the domestic compartments) by summing the DR of all species in the shared community.

Results

Dynamics of waterfowl community

Variation in waterbird numbers observed across the two years was characterized by a peak during the end of the cold-dry season and running into the hot-dry season (July-September-November; Figure 3.1). This peak resulted from two general trends: (1) the concentration of nomadic sub-Saharan waterfowl (Dendrocygnidae and Anatidae) on larger bodies of water as seasonal wetlands within the subregion dried down; and (2) the return of palearctic migrants from Europe during the (European) fall migration. The palearctic migrants leave the ecosystem between March and April, at the end of the Zimbabwean rainy season. Species diversity was highest during the dry season and lowest during the rainy season in both years. Note that there are no palearctic migrant duck species in southern Africa (Cumming et al. 2008).

Dynamics of domestic communities

The birds observed per family and the species diversity of the three domestic compartments are shown in Figure 3.2. For each of the domestic compartments, domestic species dominated the counts. Intensive poultry represented 98% of all birds observed in the intensive poultry compartment, backyard chickens represented 25% of the backyard compartment, and ostrich represented 79% of the ostrich compartment. The remaining bird community in each of the domestic compartments was quite homogenous across the

compartments, dominated by birds from the Ploceidae, Estrildidae, Ardeidae, Columbidae and Hirundinidae families, which represent between 59 and 67% of the birds observed. The maximum number of birds observed in these three communities was in April, mainly due to an increase in Ploceidae, particularly the red-billed queleas (*Quelea quelea*). This species is considered a pest species by local farmers and exhibits high variability in population dynamics. Species diversity varied between the three compartments as well as seasonally, particularly in the ostrich compartment.

Patterns of IR and MR in the waterfowl compartment and relation to the RFs

IR peaked in September 2008 and July 2009 (Figure 3.3). MR peaked in November in both years (Figure 3.4). For both risks, there was a difference in the intensity of the peak between the two years, which correlated with the variability in waterfowl abundance (Figure 3.1). The trends of all five RFs followed the MR with one major peak per year during or slightly before the dry season (Figure 3.4). The “Feeding” RFs had a slightly advanced peak in July or September depending on the year. Some RFs, such as “Mixing” and “Juvenile,” had a higher variability than others (Table 3.3). Three RFs had a significant correlation with the MR curve (Gregariousness, Abundance and Feeding, in decreasing order). The results are consistent with a higher risk of the presence of AIV strains in the waterfowl community at the end of the dry season. Waterfowl species contributing the most to IR and MR are presented respectively in Tables 3.4 and 3.5. IR for AIV and HPAI H5N1 was dominated by Charadriiformes (35.8% and 47.0%, respectively) and Anseriformes (37.1% and 33.7%, respectively). MR was largely dominated by Anseriformes.

Table 3.2: Justification and equations for introduction, maintenance and domestic risk (all RF s have previously been multiplied by the presence-absence matrix).

	RFs used and equation	Transformation	Justification
Introduction Risk (IR)	$RF1 * RF2$ For each count, for each species (no value for may 2007 due to $RF1$ calculus)	None	Each bird entering the community is associated with a AIV risk related
Maintenance Risk (MR)	$RF3 + RF4 + RF5 + RF6 + RF7$ For each count, for each species	Standardized	Each RF is additive to the others
Domestic Risk (DR)	$RF1 + RF2 + RF3 + RF4 + RF5 + RF6 + RF7$ For each species observed simultaneously in waterfowl and domestic compartment	Standardized	Each RF is additive to the other and $RF1$ & 2 represent also a risk of introduction for the domestic compartment

Table 3.3: *Standard deviation for each risk factors and the species diversity (across the values for the 12 missions) and Spearman Rank Correlation Coefficient for each risk factors and the species diversity in relation to the global risk.*

	Standard Deviation	Spearman Rank Correlation Coefficient	p value
Abundance	15,9	0,83	0,001
Gregariousness	17,7	0,87	<0,001
Mixing	26,8	0,69	0,13
Juvenile	23,6	-0,01	0,96
Feeding	10,4	0,76	0,04
Species diversity	10,2	0,35	0,258

Figure 3.1: Waterfowl community abundance per family (bars) and species diversity (blue line) across the 12 missions (encompassing 2 years).

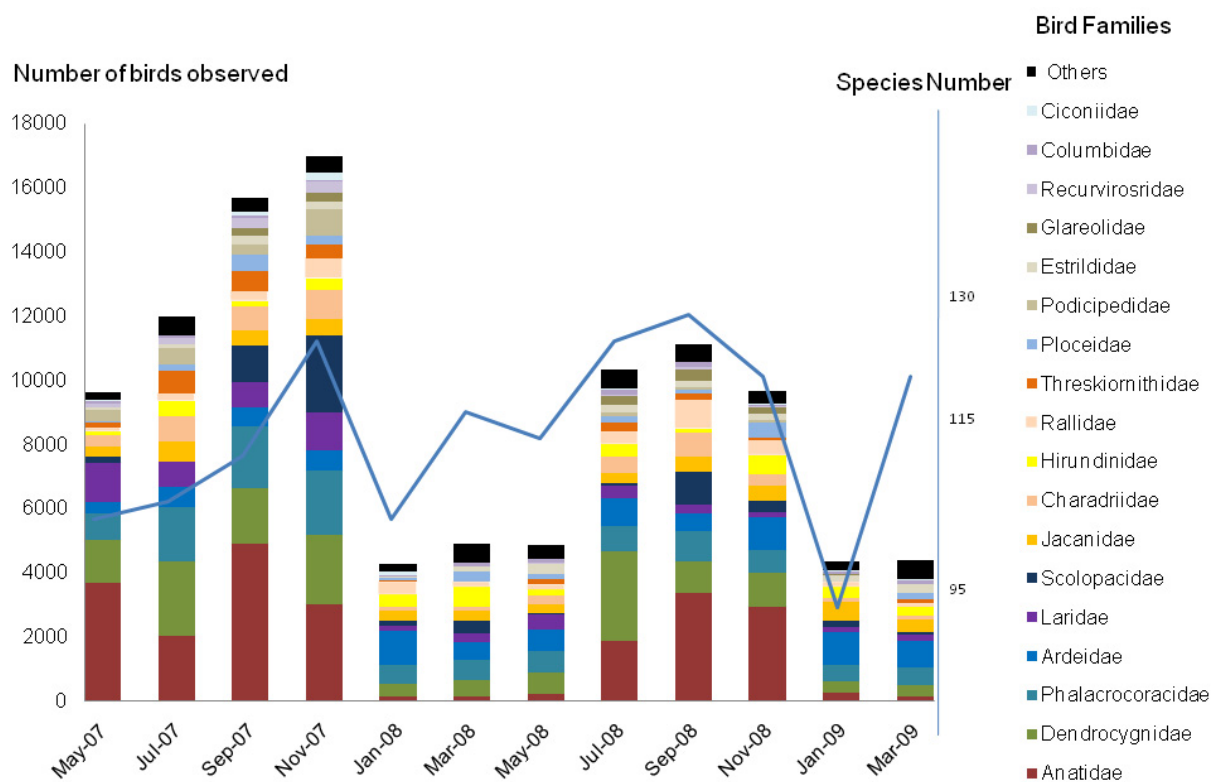
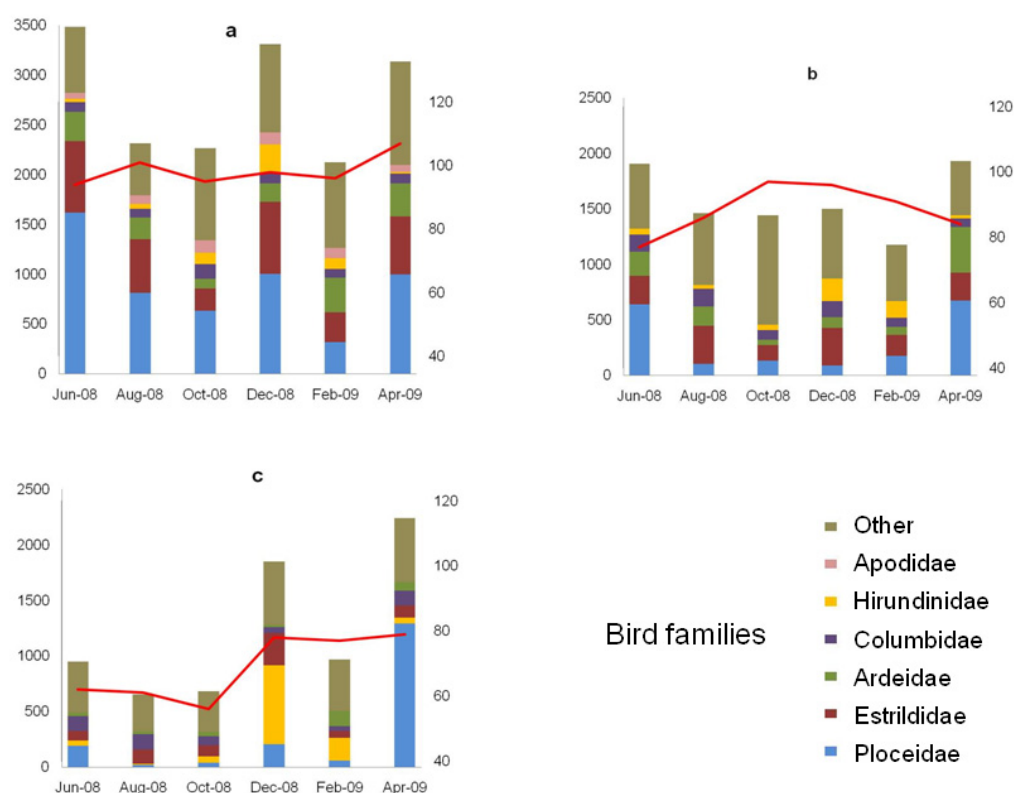


Figure 3.2: Number of birds observed per family (left axis – bars) and species diversity (right axis, red line) in a) Intensive poultry compartment (n=7 sites); b) Backyard poultry compartment (n=6 sites); c) Ostrich farm compartment (n=6 sites). This results are compiled after withdrawing the domestic species, always overrepresented in these communities (intensive poultry = 98%; backyard chicken = 25%; ostriches = 79%).



Variation in DR for the three domestic compartments

The intensive poultry and ostrich farm DR curves were similar (Figure 3.5), with two peaks of similar amplitude: one in November, the other in March. For the backyard poultry curve, only the peak in March was evident. The DR curves for the intensive poultry and ostrich compartments, and the MR curve for the waterfowl, showed the highest risk during the month of November (end of the hot-dry season). In our model, this month had the highest risk for transmission of AIV strains from the waterfowl to the domestic compartment. The second peak observed in the intensive poultry and ostrich farm DR curves was not related to a peak in the waterfowl MR. There was consistency in the most represented families for each of the three domestic compartments (Table 3.6).



Table 3.4: *Twenty most important species influencing the risk in our model ranked per Risk Factor (decreasing ranking) and sum across the 7 RFs values for the last column (RiskSum) ; (1) Abun = Abundance dynamic; (2) Greg = intraspecies mixing dynamic; (3) Mixing = interspecies mixing dynamic; (4) Juvenile = proportion of juvenile in the population dynamic; (5) Immig = difference between current and previous mission count per species dynamic; (6) Mig = movements patterns; (6) Feeding = feeding non-dynamic; RiskSum = sum of the ranks of the preceeding 7 RFs per species.*

Species	Order	Family	Relative IR for AIV	Relative IR for H5N1
Red-billed Teal	Anatidae	Anseriformes	8680	5787
Ruff	Scolopacidae	Charadriiformes	5922	5922
White-faced Duck	Dendrocygnidae	Anseriformes	5232	3488
Barn Swallow	Hirundinidae	Passeriformes	2841	2841
Unidentified wader sp.		Charadriiformes	1689	1689
Kittlitzs Plover	Charadriidae	Charadriiformes	1636	1091
Cattle Egret	Ardeidae	Ciconiiformes	1240	827
White-winged Tern	Laridae	Charadriiformes	1218	1218
Little Stint	Scolopacidae	Charadriiformes	1026	1026
Red-billed Quelea	Ploceidae	Passeriformes	964	0
Collared Pratincole	Glareolidae	Charadriiformes	865	577
Common Sandpiper	Scolopacidae	Charadriiformes	858	858
Grey-rumped Swallow	Hirundinidae	Passeriformes	757	0
White-backed Duck	Anatidae	Anseriformes	746	0
Reed Cormorant	Phalacrocoracidae	Ciconiiformes	666	0
Wood Sandpiper	Scolopacidae	Charadriiformes	648	324
Glossy Ibis	Threskiornithidae	Ciconiiformes	516	0
Red-knobbed Coot	Rallidae	Gruiformes	516	0
Egyptian Goose	Anatidae	Anseriformes	476	0
European Bee-eater	Meropidae	Corafiiformes	438	438

Figure 3.3 : Variation in the introduction risk (combined immigration and AIV risk related RFs) calculated for all AIV and for H5N1 in particular (birds potentially migrating from the northern hemisphere).

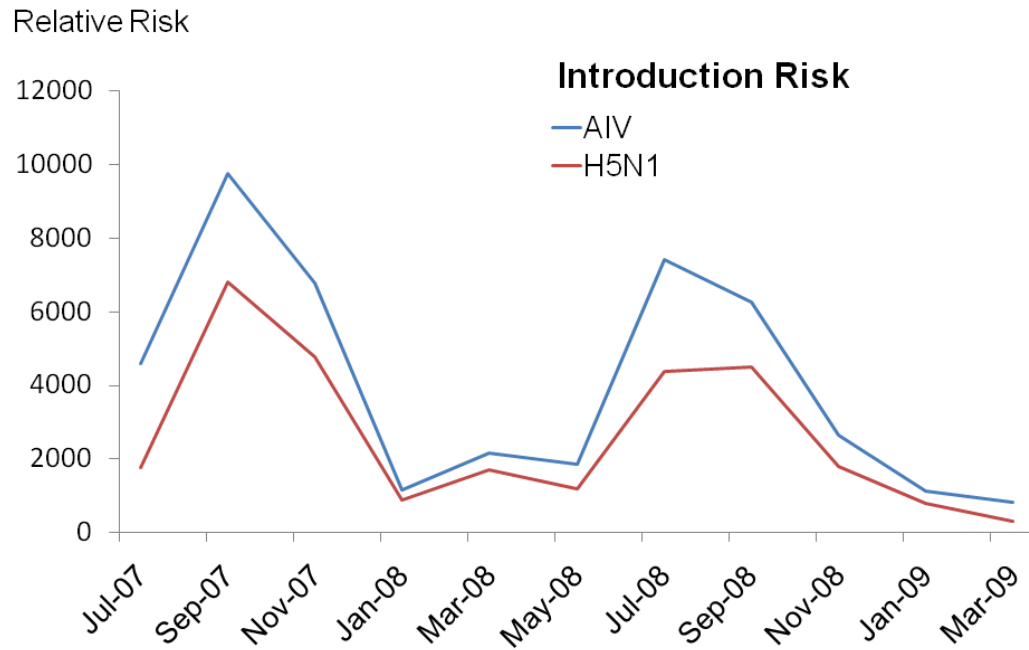
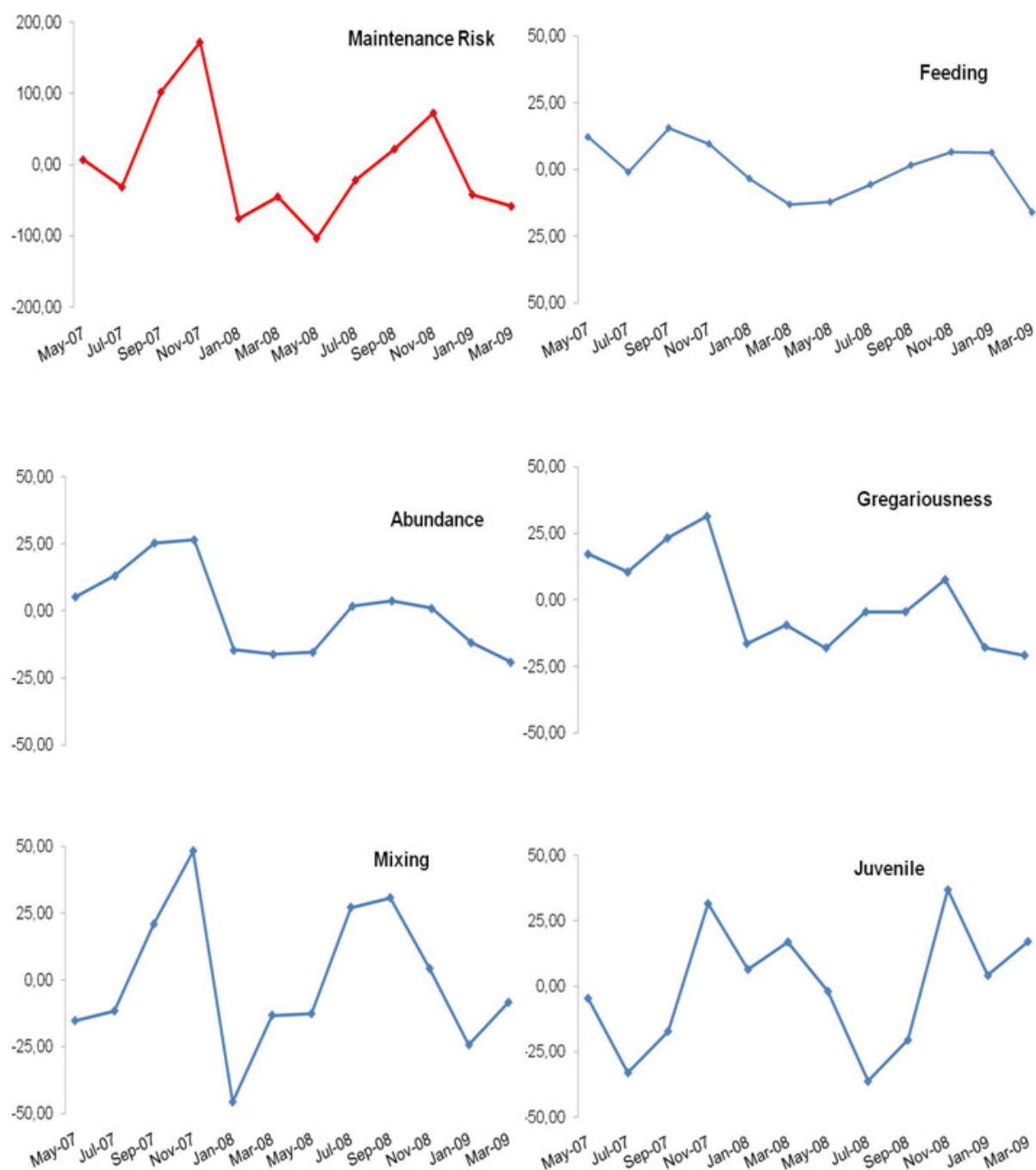


Figure 3.4: Evolution of the maintenance risk (MR) and of each RFs included in the MR in the waterfowl compartment.



Discussion

Our results provide a clear illustration of the ways in which community-level risk varies over time, both within and between years. IR peaked during the early hot-dry season, when regional waterbirds were concentrating on larger water bodies and migrants began to arrive from Europe. By contrast, MR peaked in November at the end of the dry season when the largest waterbird concentrations were observed. A number of bridge species were shared between different epidemiological compartments, suggesting a strong potential for interactions between domestic and wild birds in this system.

On the use of dynamic risk factors

We are not aware of any previous studies that have attempted to track variations in community-level risk factors through time. Although we worked primarily with indicators rather than with empirical proof of pathogen transmission, it is important to remember that community ecology and epidemiology have been used in combination for the last 25 years to explore and understand the behavior of multi-host and/or multi-pathogen systems (Holt and Pickering 1985, Hudson and Greenman 1998). A solid body of empirical evidence suggests that the availability of hosts, their movements, and their interactions with other hosts will influence pathogen transmission (Morgan et al. 2006, Bordes et al. 2009). Intra- and inter-species mixing, the presence or absence of particular species, and the proportion of juveniles in the population vary seasonally for waterfowl and are important influences on the ecology of infectious diseases (Wallensten et al. 2007). There is therefore a lot of evidence-based support for the a priori definition of RFs that take into account the ecology of hosts and the ways in which host ecology may influence the behavior of pathogens in a system. At the same

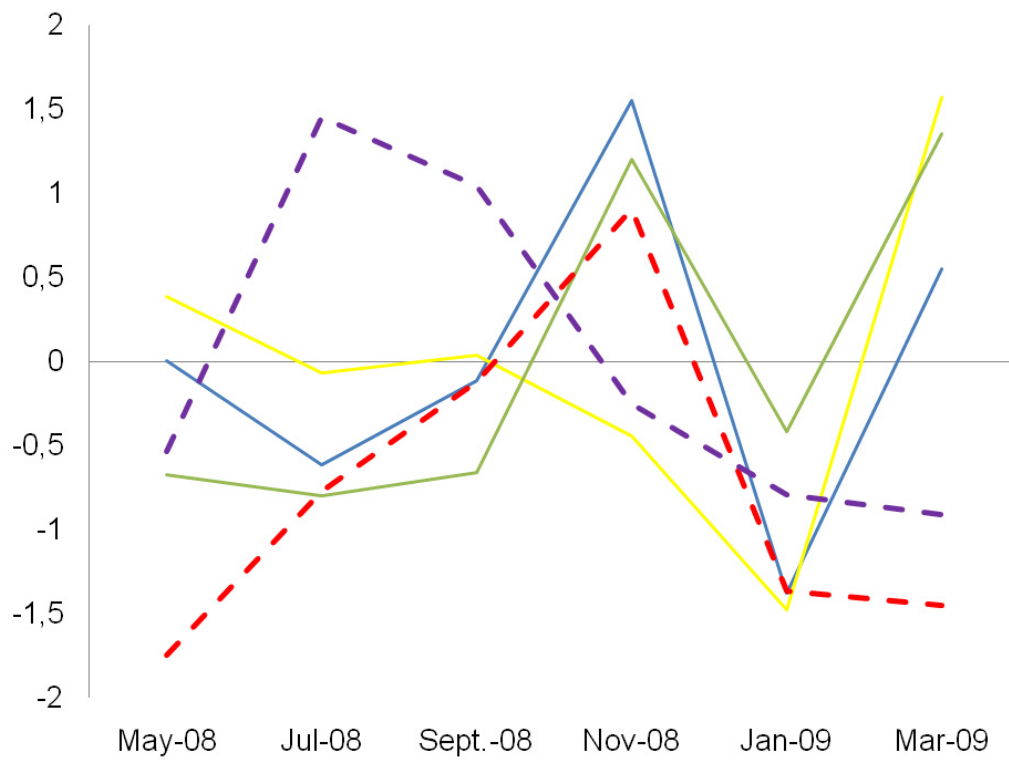
time, it is important to note that these RFs remain hypotheses until such time as further data on influenza occurrence within the system become available.

The development of dynamic RFs in previous studies has primarily focused on differences between summer and winter bird communities. Bimonthly risk mapping presents a finer-scale and considerably more informative pattern. Despite the high quality of our count data, however, a number of parameters used in this analysis remain difficult to estimate. For example, the immigration RF assumes that the arrival in the counts of new birds represents a risk for AIV introduction; in reality, numbers could stay constant while individuals change, and a proportion of the birds arriving in the system may be coming from nearby areas. For some bird species (e.g. red-billed teal *Anas erythroryncha* and white-faced duck *Dendrocygna viduata*), movement patterns are estimated from scarce ring recovery data. Often, the proportion of the population undertaking nomadic vs. trans-equatorial movements is unknown (Underhill et al. 1999). This information is important for estimating a risk of introduction (according to different AIV strains) but cannot be taken into account in our model (Cumming et al. 2008). Dispersal is particularly crucial for the two species mentioned above because they constitute some key species identified by the IR. Environmental RFs could have been taken into account in this model. In the Manyame catchment, measurements of water temperature at various seasons averaged 21.08°C (n=70; min 14.85°C; max 25.4°C, Caron, unpublished data); this supports the idea that the environment may be a potential reservoir throughout the year (with better conditions for virus survival during May-August) using data from recent studies (Brown et al. 2007b, Brown et al. 2008, Weber and Stilianakis 2008).

Table 3.5: *Twenty most important species influencing the maintenance risk in our model ranked per risk factor (decreasing ranking) and sum across the 5 RFs values for the last column (Maintenance Risk) ; A = Abundance dynamic RF; G = intraspecies mixing dynamic RF; M = interspecies mixing dynamic RF; J = proportion of juvenile in the population dynamic RF;; F = feeding non-dynamic RF; Maintenance Risk = sum of the ranks of the preceding 5 RFs per species.*

Species	Family	Order	A	G	M	J	F	Maintenance Risk
White-faced Duck	Dendrocygnidae	Anseriformes	2	3	4	1	1	11
Red-billed Teal	Anatidae	Anseriformes	1	1	9	5	1	17
African Jacana	Jacanidae	Charadriiformes	5	18	1	15	1	40
Reed Cormorant	Phalacrocoracidae	Ciconiiformes	3	7	2	6	28	46
White-breasted Cormorant	Phalacrocoracidae	Ciconiiformes	7	11	7	20	28	73
Grey-headed Gull	Laridae	Charadriiformes	4	9	5	57	1	76
Black Crake	Rallidae	Gruiformes	23	42	6	21	1	93
Red-knobbed Coot	Rallidae	Gruiformes	11	5	35	26	21	98
Egyptian Goose	Anatidae	Anseriformes	13	23	20	44	1	101
Grey Heron	Ardeidae	Ciconiiformes	17	53	3	27	1	101
Cattle Egret	Ardeidae	Ciconiiformes	9	10	13	51	21	104
Glossy Ibis	Threskiornithidae	Ciconiiformes	18	27	33	7	21	106
Black Heron	Ardeidae	Ciconiiformes	19	32	24	34	1	110
Kittlitzs Plover	Charadriidae	Charadriiformes	8	8	41	41	27	125
Common Moorhen	Rallidae	Gruiformes	31	45	18	36	1	131
Spur-winged Goose	Anatidae	Anseriformes	43	17	61	2	17	140
Yellow-billed Egret	Ardeidae	Ciconiiformes	32	63	17	30	1	143
African Sacred Ibis	Threskiornithidae	Ciconiiformes	21	25	36	33	38	153
Squacco Heron	Ardeidae	Ciconiiformes	27	62	8	56	1	154
Southern Pochard	Anatidae	Anseriformes	36	28	53	11	41	169

Figure 3.5: *Interaction Risk (DR) for each domestic compartment (plain lines, intensive poultry –blue, backyard poultry –yellow, ostric famrs-green) associated with introduction (IR) and maintenance risk (MR) for the waterfowl community (dashed lines, IR-purple, MR-red).*



MR is calculated without weighting the RFs because there is no empirical evidence from which to argue that one RF is more important than another. With suitable data collection and sampling for influenza viruses, it may eventually be possible to use linear models to weight different risk factors. Another important assumption used in this analysis is that birds seen within the counting area are potentially in contact. This assumption may not truly reflect fine-scale non-randomness in interaction networks.

Anseriformes and Charadriiformes represent the main families identified for IR, the first mainly as a function of their numbers and the second by their potential risk in introducing dangerous strains. Charadriiformes, mainly palearctic waders, but also Anseriformes crossing the equator are identified by the model as potential introducers of HPAI H5N1. Interestingly, when waterfowl are ranked for each of the five RFs and the ranks are summed across the two years, the species contributing the most to the MR (Table 3.5) belong to the bird orders known to be reservoirs for LPAI strains (Anseriformes and Charadriiformes) with the two most influential species in the model, the white-faced duck and the red-billed teal, being the most abundant ducks in the system. The only other orders present in the 20 most important species were Gruiformes (Coot sp.) and Ciconiiformes (Cormorant, Egret and Ibis spp.). These orders and families have been found with, or dead of, LPAI or HPAI strains (Gauthier-Clerc et al. 2007, Hars et al. 2008, Stoops et al. 2009). Additionally, the MR curve (Figure 3.4) was consistent across the two years and indicated a maximum risk of AIV presence in the waterfowl community during the hot-dry season, when migratory and palearctic waterfowl are present in the system, coming from areas where AIV strains circulate. This result is consistent with a basic epidemiological model for AIV in Africa (Appendix One - Gaidet et al. 2007) that assumes a strong likelihood of introduction of strains during the palearctic migration. The fact that most of the RFs follow the MR trends reflects some consistency in the model: the high risk season for AIV presence in the waterfowl community derives from a convergence of

peaks of RFs during this season. The “Gregariousness” and “Abundance” RFs have a high correlation with MR and an increase in the weight of these factors would accentuate the current trend in MR (Table 3.3).

The difference in MR between the two years reflects the differences in bird abundance. There is a relationship between lake level (determined by the rainfall in the previous year and human management) and bird abundance; the lakes dry down during the hot-dry season and exposed shorelines offer a muddy, vegetated, resource-rich habitat for dabbling ducks and waders. MR defined here could be predicted in advance with rainfall from the previous years, offering the potential for disease forecasting in this system. The use of environmental data to predict epidemiological patterns through an ecological (host or vector) link have already been demonstrated (Harvell et al. 2002).

Domestic Risk (DR) between waterfowl and domestic compartments

The trends in the DR curves for the three domestic compartments were different. The 19 domestic sites chosen varied between zero and ten kilometers from the lake shore, and this distance could have influenced the observed wild bird community. However, although the ostrich farm sites were the farthest from the lake shore, their DR curve followed the intensive poultry DR curve. There may be other factors besides distance to the lake that influence the wild bird community, including variation in artificial resource availability in the production buildings, farms and villages; natural resource availability; breeding sites; predation; and so on. The most likely explanation for the similar trends between intensive poultry and ostrich farm DRs is that they both used artificial feed, attracting specific bird communities, while backyard chickens forage for their own food like wild birds.

Table 3.6: *Most important families (% of the total shared community between domestic and waterfowl compartment in birds observed) participating to the epidemiological interaction defined as domestic risk (DR) between the waterfowl and each of the 3 domestic compartments during peak risk period; in the last column, the most representative species of these families (% of the number of birds observed for this family)*

Intensive Poultry	November Peak	Mars Peak	Representative Species
Ploceidae	30,30%	31,80%	Red-billed quelea (77%)
Estrilidae	21,80%	18,60%	Bronze mannikin (50%)
Hirundidae	8,50%	0,00%	Barn swallow (90%)
Ardeidae	0,00%	10,60%	Cattle egret (85%)
Total	60,60%	61,00%	

Backyard Poultry	May Peak	Mars Peak	Representative Species
Ploceidae	33,40%	34,80%	Red-billed quelea (89%)
Estrilidae	13,50%	13,10%	Bronze mannikin (52%)
Ardeidae	11,50%	21,10%	Cattle egret (97%)
Total	58,40%	69,00%	

Ostrich Farm	November Peak	Mars Peak	Representative Species
Hirundidae	38,30%	0,00%	Barn swallow (99%)
Estrilidae	15,90%	9,30%	Bronze mannikin (60%)
Ploceidae	11,20%	57,50%	Red-billed quelea (80%)
Columbidae	0,00%	5,80%	Cape Turtle Dove (76%)
Total	65,40%	72,60%	

IR was not related to any peak of the DR. However, according to our model, there are always interactions between the waterfowl and domestic compartments. In a specific epidemiological situation (e.g. regional spread of a HP strain threatening the ecosystem), this IR could help to target surveillance and control measures during high interaction seasons. The fact that the highest DR curve for two domestic compartments coincided with the highest waterfowl MR is of interest (Figure 3.5). The end of the hot-dry season is a high risk period for these two domestic compartments, not only because the waterfowl community has the highest risk of harboring AIV strains but also because the epidemiological interactions between the compartments are at their highest. We can hypothesize that this period represents a hotspot for pathogen circulation and transmission between compartments (Jones et al. 2008). The second peak after the end of the rainy season (in March) was consistent for the three domestic compartments but was not linked with a peak in risk associated with the waterfowl community. However, the shared community of wild birds between the waterfowl community and the three domestic compartments was always high (Table 3.6) suggesting a year-long risk of pathogen transmission from the waterfowl compartment. The validity of the DR estimate is limited by its population-level approach; birds of the same species observed in two different compartments were assumed to belong to the same population. However, we cannot prove that they were indeed the same individuals beyond the fact that the study site is fairly small.

Validating the model and testing the bridge species hypothesis

In order to validate the global approach and the RFs used, long-term and intensive monitoring of waterfowl will be necessary. Community analyses based on bird census data, as presented here, can contribute to the development of specific hypotheses relating to AIV

maintenance and spread in the system. The community level perspective is often missing in multi-host wild population studies (Yasue et al. 2006). Usually, access to wild individuals is difficult, technically biased or limiting, and for most capture protocols it is not possible to choose precisely the epidemiological sample composition and size (Wobeser 2002). By contrast, as this study demonstrates, bird count data can drive the sampling design and/or provide an indication of the representativeness of the samples obtained from the system.

To test hypotheses concerning the role of bridge species between waterfowl and the domestic compartments usually requires selective sampling among a broad range of avian diversity. More than 100 species in 25 families of birds have been detected dead or alive with AIV strains (Olsen et al. 2006). Some terrestrial birds have been found to harbor AIV strains and even HPAI H5N1 strains (Nestorowicz et al. 1987, Boon et al. 2007, Brown et al. 2009). We thus assumed that any wild bird species could be capable of harboring and transmitting AIV strains. Consideration of the families and species contributing the most to the peak DR for each domestic compartment (Table 3.6) shows that the first four families represent between 58% and 72% of the total of birds involved in the DRs. For each of these families, there is one species that represents between 50 and 99% of the birds observed. This unexpected result means that only a few species represent the bulk of the DR and that a targeted sampling focusing on these species will achieve not only a surveillance of the species most at risk of transmitting AIV but also an extensive coverage of the overall DR. Sampling protocols targeting these species should cast light on the role of potential bridge species between the waterfowl and domestic compartments. To our knowledge, there has not been sufficient local-scale testing of potential bridge species to characterize a bridge species community, despite some published suggestions (Veen et al. 2007) and an obvious missing link in HPAI outbreaks that have involved spatially segregated poultry and waterfowl.

Conclusions

The ultimate goal of this study was to integrate ecological and epidemiological data in a risk-mapping context (as discussed in Chapter Two - Caron et al. 2009). The main outputs are a set of hypotheses that describe the mechanisms that generate patterns of AIV circulation in the waterfowl community and the role of bridge species between the waterfowl and the domestic compartments. Although we have focused on a one-way analysis (from the waterfowl compartment to each of the domestic compartments), the same analysis could be conducted for transmission between the four compartments in both directions.

An important advantage of our sampling protocol is that it provides the information that is needed to assess the adequacy of epidemiological sampling. This step is often missing in wildlife surveillance and decreases the validity of results. The next step will be to add to this data set an AIV prevalence layer (i.e., of wild and domestic compartments) to test the model and the bridge species hypotheses. The protocol described here is intensive but feasible. Its approach could easily be simplified and reproduced. In the context of AIV surveillance, a series of counts by ornithologists during suspected high-risk seasons would prepare the ground for targeted sampling. In some countries, this type of data is regularly collected by ornithological organizations and is therefore already available.

The strength of this research relative to traditional epidemiological analyses lies in its ecological dimensions. Although our model was designed with the ecology of AIV in mind, most pathogens with direct transmission will be dependent on the ecological traits estimated by the RFs (with some adjustments; e.g. “Feeding” RF). Can this risk factor analysis be extended to other pathogens to develop more ‘ecological’ predictions of disease risk? Such approaches may ultimately provide useful guidelines for surveillance in hotspots of disease emergence at the wildlife/domestic interface (Jones et al. 2008).

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Chapter Four: Persistence of Low Pathogenic Avian Influenza Virus in Waterfowl in an African Ecosystem

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115)



Introduction

Low Pathogenic Avian Influenza (LPAI) viruses in the Northern Hemisphere are maintained by their waterfowl reservoirs – mainly Anseriformes - and the environment (Webster et al. 1992, Olsen et al. 2006). Waterfowl can provide a source of LPAI strains for domestic avian populations, in which they can evolve into Highly Pathogenic AI (HPAI) (Abolnik et al. 2007, Abolnik et al. 2009, Chapter Two - Caron et al. 2009). Previous studies have established the presence of LPAI in palearctic migrants and afro-tropical birds in Africa, but no maintenance mechanism has been described (Abolnik et al. 2006, Appendix One - Gaidet et al. 2007). *A priori*, the high temperatures experienced by afro-tropical regions should decrease the potential survival of the virus in the environment and hence prevent the persistence of LPAI throughout the year (Brown et al. 2009). In this study we present results from a longitudinal survey of LPAI in waterfowl conducted in Zimbabwe in 2007-2009 to test their potential persistence in an African ecosystem.

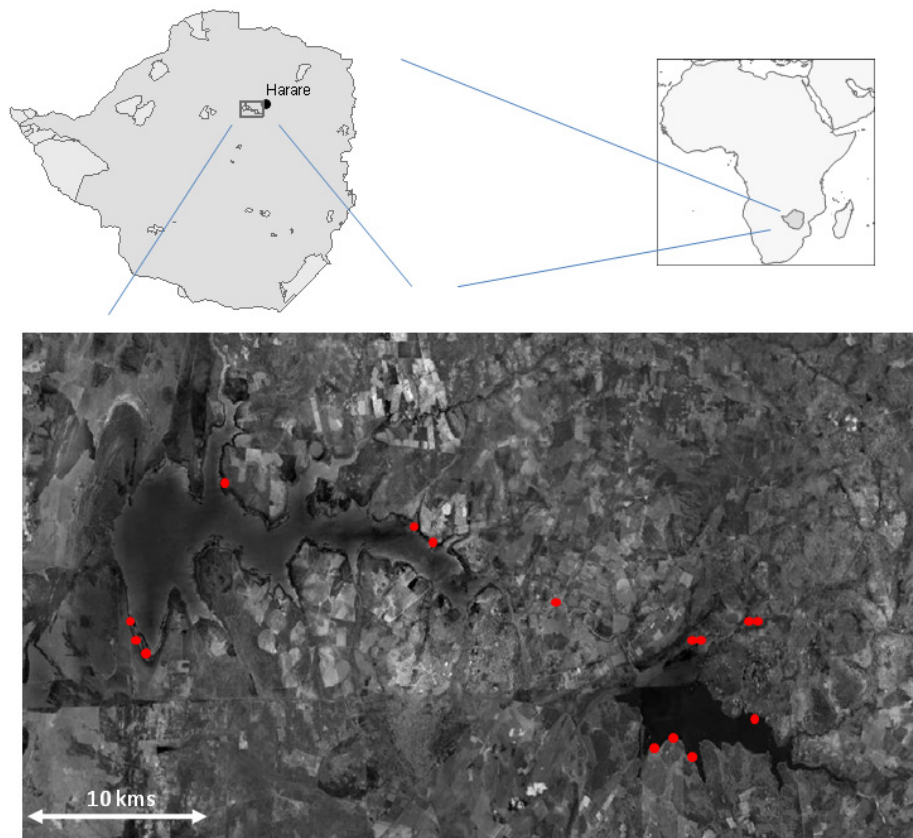
Methods

The study was undertaken in the Manyame catchment (E30°30'30'', S17°45'45''), 35 kilometers West of Harare, on two adjacent lakes (Lake Chivero and Manyame, 65 and 185 km² respectively)(Figure 4.1). Both lakes are important waterfowl habitats in Zimbabwe. Although palearctic Anseriformes seldom reach this area during their migration, it hosts a range of other palearctic waterbirds as well as passerines and raptors during September-April (Figure 4.2a). In addition, Afro-tropical nomadic and resident species inhabit these lakes. Waterfowl were counted and captured every two months between May 2007 and March 2009, resulting in 12 count and capture sessions encompassing 2 years. Fifteen representative sites

were selected using local ornithological expertise (Figure 4.1). For each session, 4 point counts were done in each site at different times of day. Counts lasted half an hour and included all birds within a 150m radius. After a week of site preparation (baiting), captures were performed during a week in suitable counting sites using baited walk-in traps and mist-nets. For each bird captured, cloacal and tracheal swabs were collected, placed in viral transport medium (phosphate-buffered saline/glycerol/ antibiotics), and transported in liquid nitrogen prior to testing. RNA was extracted using the MagNaPure LC total nucleic acid isolation kit-high performance (Roche, Mannheim, Germany) on a MagNAPure robot (Roche). Real-time reverse transcription PCR was performed using the VLA TaqMan® Influenza A/H5/H7 Detection Kits (Applied Biosystems) on either a StepOnePlus (Applied Biosystems) or a Light Cycler 480 (Roche) platform. Positive samples were inoculated into embryonating fowls' eggs for virus isolation according to standard procedures (OIE 2008).



Figure 4.1: Counting and capture sites (red dots) in Manyame (left) and Chivero (right) lakes with location of the ecosystem in Zimbabwe (light grey areas represent national parks of Zimbabwe) and of Zimbabwe in Africa. Lakes' satellite pictures were obtained through Google Earth database (® Google Earth).



Results

A total of 1601 waterbirds were captured. The number of birds captured averaged 133 per session (with a minimum of 61 and a maximum of 247) (Figure 4.2b). These captures represented 96 species. Anseriformes constituted 46.8% of captures, and were dominated by 2 species, viz. *Anas erythrorhyncha* (red-billed teal, 66.5%) and *Dendrocygna viduata* (white-faced whistling duck, 26.9%). The composition of bird sampled per order is reported in Table 4.1.

This is the first report of the persistence of LPAI strains over a year in waterfowl in an African wetland. LPAI strains were detected in the waterfowl community during 10 consecutive sessions over a period of 20 months with a prevalence ranging from 1.3-22.3% (Figure 4.2c). Of 2791 (51.1% cloacal, 48.9% tracheal) test results, 100 samples were positive for the presence of RNA of the influenza A virus group (95 birds, 5.9% of total of birds), 9 (0.6%) and 10 (0.6%) were positive for the H5 and H7 subtype respectively (Table 4.2). 49.5% of positive birds were ducks, of which 93.7% were *Anas erythrorhyncha* (Anatidae) or *Dendrocygna viduata* (Dendrocygnidae). Global prevalence per session varied between 0.0 and 22.3% across species and between 0.0 and 20.0% for Anseriformes (Figure 4.2c). No viruses could be isolated.

Table 4.1: Sample size per session and proportion of swab sampled per order. The second row indicates the number of swabs (cloacal or tracheal) tested per session. The following rows indicate percentage of swab belonging to different bird order.

	May-07	Jul-07	Sep-07	Nov-07	Jan-08	Mar-08	May-08	Jul-08	Sep-08	Nov-08	Jan-09	Mar-09	Total
Swab results (c+t)	n=200	n=279	n=414	n=161	n=227	n=163	n=216	n=218	n=271	n=356	n=194	n=92	n=2791
Anseriformes	66.0	87.8	79.0	79.5	16.3	37.4	59.3	41.3	16.6	33.7	5.7	22.8	48.2
Charadriiformes	29.0	10.8	19.1	17.4	60.4	27.6	16.7	43.6	52.0	48.3	67.5	33.7	35.2
Passeriformes	1.0	0.7	1.4	0.6	16.3	26.4	10.2	9.2	17.7	12.4	7.2	8.7	8.8
Columbiformes	0.0	0.7	0.0	0.0	0.0	2.5	4.6	3.2	3.0	1.4	9.8	10.9	2.3
Coraciiformes	1.0	0.0	0.0	1.2	6.6	3.7	3.7	0.0	5.2	1.1	1.0	10.9	2.3
Ciconiiformes	1.0	0.0	0.0	0.0	0.4	1.2	0.9	0.0	4.8	2.2	6.7	8.7	1.8
Divers	2.0	0.0	0.5	1.2	0.0	1.2	4.6	2.8	0.7	0.8	2.1	4.3	1.4

Table 4.2: Prevalence (prev; in percent), sample size (n) for the entire birds sampled (All birds) and only the ducks sampled (Ducks Only) compared between sessions when palearctics birds are present in the ecosystem (September to March) and absent (May to July). Results for Chi-2 test and level of significance, testing the null hypothesis H_0 : $Prev(May-July)=Prev(Sept-Mar)$.

	May-July		Sept-Mar			
	prev	n	prev	n	Chi-2	p
All birds	2,63	494	7,23	1106	13,19	<0,001*
Ducks Only	3,76	319	8,14	430	5,94	<0,01*

Table 4.3: PCR positive swabs for Avian Influenza and hemagglutininase type when available. The second row indicates the number of swabs positive per session. The following rows indicate the number of swabs tested PCR positive for Avian Influenza: the hemagglutininase type is determined (H5 or H7) or not available (na).

	May-07	Jul-07	Sep-07	Nov-07	Jan-08	Mar-08	May-08	Jul-08	Sep-08	Nov-08	Jan-09	Mar-09	Total
Swab positive	p=5	p=2	p=26	p=5	p=34	p=4	p=4	p=2	p=2	p=16	p=0	p=0	p=100
Anseriformes	4*na 1*H7	2*na	17*na 2*H7	5*na	3*na 3*H5	2*na	4*H7	2*na	1*na	3*na	0	0	39*na 3*H5 7*H7
Charadriiformes	0	0	5*na 1*H7	0	14*na 4*H5 2*H7	2*na	0	0	0	8*na	0	0	29*na 4*H5 3*H7
Passeriformes	0	0	1*na	0	5*na 1*H5	0	0	0	1*na	5*na	0	0	12*na 1*H5
Coraciiformes	0	0	0	0	1*na 1*H5	0	0	0	0	0	0	0	1*na 1*H5

Discussion

In both years, viruses were detected in the waterbird community during the period when palearctic birds are absent or rare (May-July sessions). This result suggests the yearly persistence of LPAI in Afro-tropical waterfowl and raises the hypothesis of an endemic cycle in Zimbabwe or at least in Southern Africa. However, Table 4.2 indicates higher prevalence when the palearctic birds are present in the ecosystem (mainly from September to January) compared to when there are absent (May to July). Therefore, AI viruses (AIV) appear to be present all year long, although we cannot exclude the necessity of seasonal introduction of AIV by palearctic birds in September to maintain the cycle. Observing viral persistence, we cannot prove maintenance of LPAI in this ecosystem.

The detection of subtypes H5, H7 and other influenza A viruses (subtypes undetermined) in ducks indicates the simultaneous circulation of multiple subtypes. The H7 subtype was detected during 14 consecutive months, although not for each session (4 out of 12 sessions) during both dry and wet season and on two consecutive years. From these data one cannot deduce that the H7 or H5 subtypes detected in subsequent years were related. However, the results do suggest that, at least for some subtypes, some strains may be maintained throughout the year and between years. The H5 subtype was detected only during the January 2008 session (Table 4.3) but in a relatively high prevalence: H5 does not appear to be a common H subtype in waterbird communities (Krauss et al., 2004, Wallensten et al. 2007).

The high prevalence that we observed during the 2007 hot-dry season (September-November) and beginning of the rainy season (January 2008) is comparable to the prevalence level reported in sites in the Northern hemisphere during fall migration (September) (Krauss et al. 2004, Wallensten et al. 2007). The seasonal variations in prevalence have to be

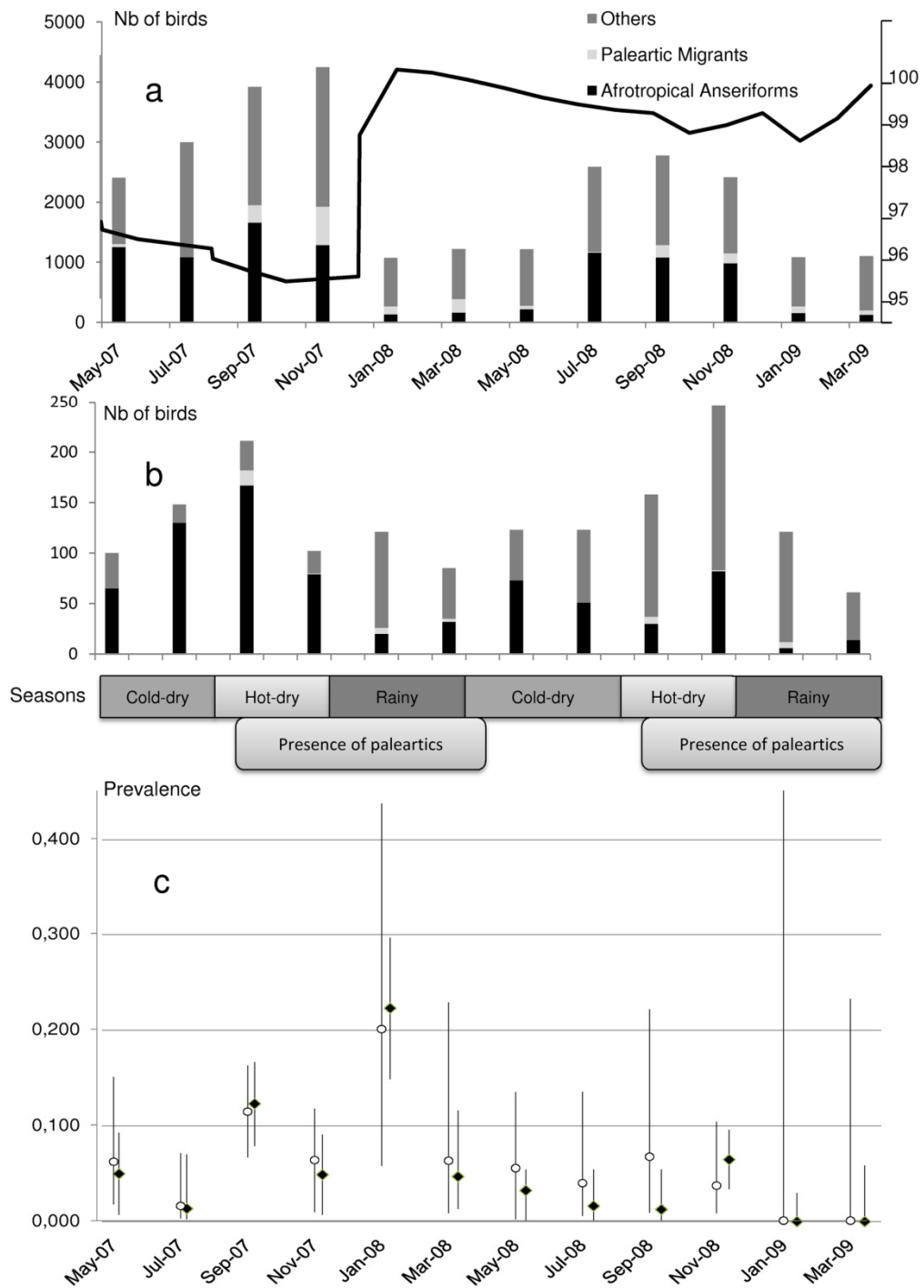
interpreted with caution as the sample composition in species and abundance varied between sessions, due to capture bias (Figure 4.2b). For example, January 2008 and 2009 report the highest and lowest densities both with small sample size. However the profile of Anseriformes' prevalence (controlling for part of this bias) is similar in trends and intensity to global prevalence. A small sample size of ducks was always associated with small numbers of ducks counted (Figure 4.2 a, b). During the hot-dry season, ducks tend to concentrate in lakes and these flocks provide ample opportunity for disease transmission. During the rainy season (December-Mars), ducks tend to disperse out of the study lakes to breed; our sample size reflects these movements. During the first rainy season (January 2008), viruses were circulating despite few ducks in the system (4 ducks out of 27 positive birds), suggesting a complementary role of local and palearctic Charadriiformes and Passeriformes in the persistence process (Table 4.1 & 4.3).

Environmental conditions in this ecosystem (1500m altitude and an average annual temperature of 17.9°C) are typical of Eastern and Southern African highveld and could be compatible with virus survival in the water (Stallknecht et al. 1990). We failed to detect any positive samples during the 2009 rainy season. This result may be explained by a small sample size or by the non-persistence of LPAI strains in this ecosystem. The observed concentration of afro-tropical ducks on the lake shores during the hot-dry season is driven by a combination of increased resource availability on receding lake shores (Figure 4.2a), the complete drying-up of non-perennial wetlands leaving in the ecosystem a few water bodies to be occupied by the same bird community, and the need for the birds to undergo flightless moult in a deepwater location. This influx of waterbirds into the ecosystem offers numerous opportunities for viral introductions from other African regions (afro-tropical nomadic species) or from Eurasia (palearctic migrants) (Abolnik et al. 2006). In addition, this concentration of hosts during the hot-dry season when the viruses should have the lowest

survival in the environment may contribute to viral maintenance: the high density of birds provides higher host availability and higher rate of contact between hosts, decreasing the time necessary for successful fecal-oral transmission. The seasonal aggregation of ducks in the Manyame catchment could explain the higher prevalence observed during the first hot-dry and early rainy season. The combination of favorable environmental conditions during particular seasons (rainy and dry-cold season) and the presence of palearctic birds and high duck density during harsh season (hot-dry) could support a persistence or maintenance hypothesis in this ecosystem, although we cannot resolve the effects of specific factors. Environmental transmission has been shown to play an important role in viral persistence in stochastic models of disease transmission (Breban et al. 2009, Rohani et al. 2009).

The observed inter-annual variation (including the non-persistence in 2009) could be explained by variability in environmental factors. Rainfall determines lake levels and triggers a differential waterfowl concentration during the following hot-dry season. This hypothetical relation can be observed in Figure 4.2a: a low lake level is associated with a high bird concentration in 2007, in contrast to 2008. Viral persistence (driven by host availability and susceptibility) could thus be dependent on waterfowl density. Our data could be interpreted as showing that during the hot-dry season of 2008, the threshold density was not reached and the LPAI did not persist.

Figure 4.2: (a) Birds counted per session: in black duck species, in light grey palearctic migrants and in dark grey other species; the solid line (linked to the right vertical axis) represent the variations of the lake level; (b) birds captured per session: in black duck species, in light grey palearctic migrants and in dark grey other species; (c) global (black dots) and duck (white dots) prevalence (%) per session with confidence interval. An indication of the seasons in this ecosystem is given in blocks and the period when palearctic migrants are present in the system is also presented.



Conclusion

In conclusion, our data suggest the persistence of multiple AIV strains in the waterfowl community of a Zimbabwean ecosystem for 20 consecutive months. The persistence of LPAI in an African ecosystem indicates that the role of afro-tropical ducks in LPAI epidemiology requires further assessment. This study suggests that African ecosystems are not merely passive receptors of AIV from Eurasia. African waterfowl communities have the potential to harbor multiple viral strains for an extended period, indicating that they play a broad-scale role in the epidemiology of AIV. The recent detection in Nigeria of HPAI H5N2 in apparently healthy waterfowl (including *Dendrocygna viduata*) reinforces this hypothesis (Gaidet et al. 2008) with phylogenetic data linking this strain to European and African waterfowl. A relation between environmental determinants, host community ecology and virus ecology is presented with potential for a predictive approach. These observations are particularly relevant for animal and public health at the wildlife/domestic/human interface in two contexts: (a) in Africa, where HPAI H5N1 is recurring in domestic poultry production systems (Ducatez et al. 2007, Cattoli et al. 2009) (providing opportunities for recombination with local LPAI), veterinary/public health sectors may be weak, and the wild/domestic bird interface is rarely monitored or controlled despite a predicted risk (Kilpatrick et al. 2006); and (b) in Eurasia, since the spring migration of palearctic birds can theoretically expose Eurasian ecosystems to LPAI strains from Africa (although thus far, with little information available, no genes of African origin have been detected in the Eurasian viral pool) (Abolnik et al. 2006, Abolnik 2007).

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Chapter Five: Risk of diffusion of a Highly Pathogenic Avian Influenza virus between wild and domestic avian compartments through bridge species in Zimbabwe

(Chapter reference: Caron, A., Grosbois, V., Etter, E., de Garine-Wichatitsky, M. (In Prep)

Risk of diffusion of a Highly Pathogenic Avian Influenza virus between wild and domestic avian compartments through wild bird movements in Zimbabwe)



Introduction

The panzooty of Highly Pathogenic Avian Influenza (HPAI) H5N1 and its threat to poultry and human health (Webster et al. 1992, Olsen et al. 2006, Webster et al. 2006) have raised concerns about the role of wild birds in the ecology of Avian Influenza viruses (AIV). Waterfowl (Anseriformes and Charadriiformes) are considered reservoir for Low Pathogenic AIV (LPAI) strains but the massive HPAI H5N1 outbreaks in waterfowl tend to dismiss these species as reservoir for HPAI (Wang et al. 2008) although this role cannot be confidently ruled out. Wild birds could also be involved in the epidemiology of AIV through their potential to spread these viruses. Phylogenetic analyses of isolated strains indicated inter-continental movements of strains across wild bird populations (Koehler et al. 2008). Gaidet et al. (2008) followed the movements of a HPAI H5N2 infected healthy waterfowl across international borders. If the consequences of AIV infections on migrating bird is still discussed (van Gils et al. 2007, Arnoe et al. 2011), the study of individual bird movements or waves of migrating populations in relation to the epidemiology of AIV in wild birds (Gaidet et al. 2010) indicates that these hosts play a role in medium and large scale spread of LPAI and possibly HPAI (Wang et al. 2008, Reperant et al. 2010).

At a finer spatial scale, little is known about the role of wild birds as potential spreaders of AIV virus (Veen et al. 2007). This role is however crucial in linking the AIV natural reservoir to the avian production sector. Risk-based approaches for the local spread of HPAI have identified wild birds as a risk factor for HPAI transmission (Gilbert et al. 2006, Tiensin et al. 2009). As the spatial scale changes, the criteria for eligibility of wild bird species as spreaders of AIV also differ. A bird excreting the virus for a few hours and involved in small-scale movements could spread the pathogen without having to consider the ecological fitness issue at this spatio-temporal scale. Under these circumstances, the range of eligible spreader species increases vastly and approaches the size of the local avian species richness.

Once the virus is introduced in an ecosystem, it can spread through the movements of wild birds and infect naive bird populations through contacts with infected populations. Species spreading a pathogen from an infected to a naive population are termed “bridge species”. Throughout this article, the term “bridge species” will be used for any bird species with the potential to spread AI strains from an infected bird compartment, defined here as “a set of avian populations under similar environmental drivers” (Figure 5.1, Chapter Two - Caron et al. 2009) to a naive bird compartment; as a consequence, any waterfowl species can in principle be eligible as bridge species when they are in contact with birds of a naive compartment. Despite experimental infection trials (Brown et al. 2009, Fujimoto et al. 2010, Nemeth et al. 2010) and field sampling (opportunistic sampling when targeting waterfowl species), the capacity of potential bridge species to locally spread HPAI between bird populations, particularly at the wild/domestic interface is still largely unexplored.

The selection of potential bridge species out of the available avian diversity in an ecosystem is difficult. Caron et al. (2010) (Chapter Three) presented a framework for this selection process. In order to adequately study the role of bridge species in transmitting AIV in-between bird populations of interest, the epidemiological interaction (EI) concept is used (Chapter Three - Caron et al. 2010). Here the EIs are estimated using bird census data. The co-occurrence of bird species at different counting points during the same period are used to estimate the potential EIs within and between these counting points. At the ecosystem level, the co-occurrence of birds from the same species at different focal points and at the same time suggests potential contacts. This approach assumes that all bird species are potential bridge species, since there is limited data available on the susceptibility for AIV for most bird species.

In this article, ecological and epidemiological data are integrated to provide a risk analysis of the spread of a HPAI virus from an infected bird population to naive bird populations. In an

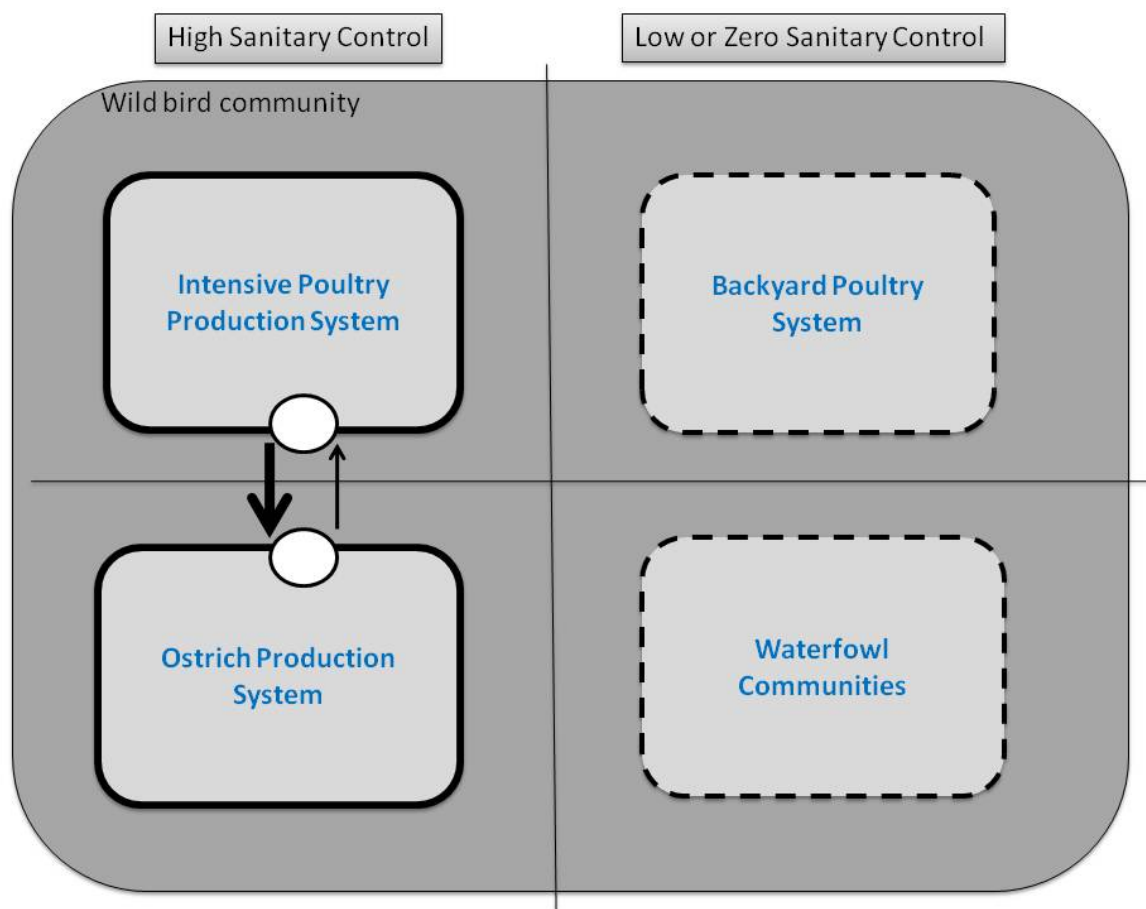
African ecosystem with four bird “compartments”, we use bird census to construct the most likely spread pathways and identify most probable bridge species for spreading a HPAI to naive avian compartments.

Materials and Methods

Study site

Lake Chivero and Manyame in the Manayme river catchment in Zimbabwe are two artificial dams built in the 50's to supply water for Harare, capital of Zimbabwe (approximate GPS coordinates: E30°30'30'', S17°45'45''). Chivero and Manyame lakes cover an area of 65 and 185km² respectively, although they experience substantial variation in their water surface areas due to the seasonality of rainfall and artificial water management by humans. The study site encompassed the area in a 10km radius around the lakes. Waterfowl living on lakes shore were defined as a bird compartment, considering that natural conditions define a common selective environment. In the direct periphery of the lakes, ostrich farms, intensive poultry farms and traditional backyard poultry systems existed and were defined as three additional compartments. We considered wild bird communities (distinct from the waterfowl compartment, which is restricted to water-dependent species) surrounding the four compartments as the source for potential bridge species able to spread the virus from one compartment to another (Figure 5.1).

Figure 5.1: Conceptual representation of the study site using compartments (light grey rectangles with border line representing the intensity of sanitary control in the compartment) and the wild bird community (large dark grey rectangle encompassing all compartments) in which each compartment is embedded. In order to illustrate the method, two counting sites are represented as circles and potential epidemiological interactions estimated from these counting sessions are shown as arrows between compartments.



Counting protocols

Wild bird counts in each site were implemented according to a protocol previously described (Chapter Three - Caron et al. 2010, Appendix Five - Cumming et al. 2011). Focal count points of thirty minutes were implemented four times every two months during one year (May 2008 – May 2009, n=6 sessions) in 15 waterfowl sites, and in 6, 6 and 7 sites in respectively backyard poultry, Ostrich farms and Intensive poultry compartments. This first protocol will be later called the “intensive protocol”. A protocol restricted to 7 waterfowl sites, 2 backyard poultry and 2 intensive poultry sites was repeated from September 2009 to November 2010, during 8 counting sessions at two months interval. This “longitudinal protocol” encompassed 14 sessions from May 2008 to November 2010. Counting sessions in waterfowl compartments were associated with the closest domestic counting sessions in time to calculate variables related to the shared community of wild birds.

Questionnaires in the three domestic production systems were conducted with managers in order to understand the population dynamics in each of these three compartments and to calculate in each compartment the number of infected (n_i) or susceptible (n_s) hosts (questionnaire data not shown).

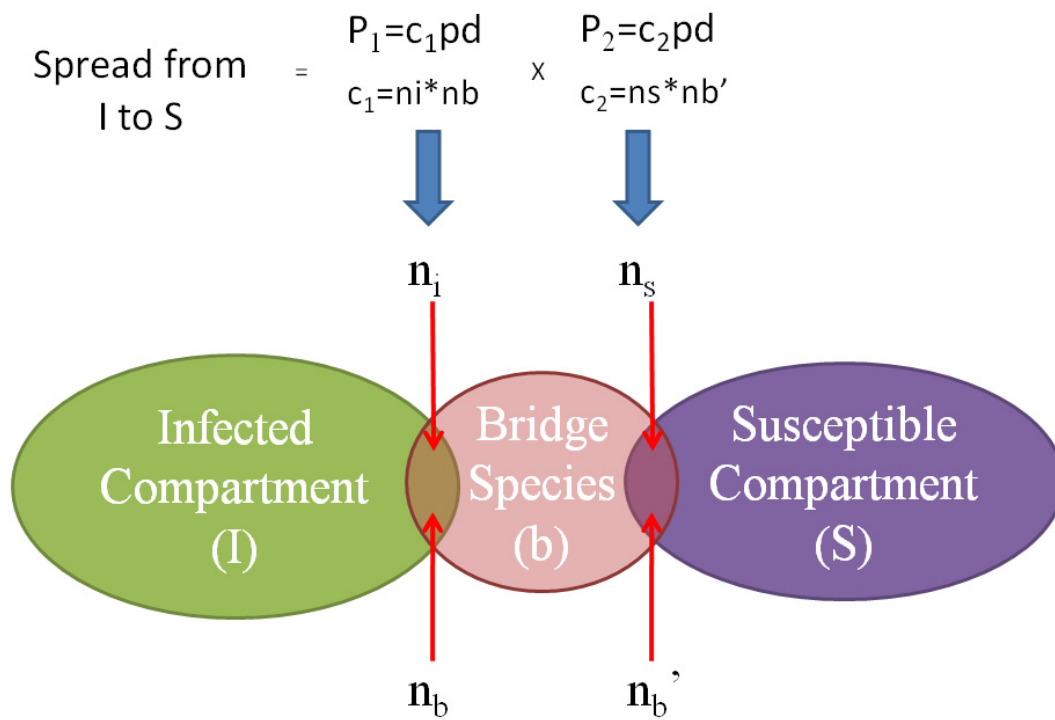
Estimation of epidemiological interactions (EIs)

EIs were estimated by using the shared community of wild birds (potential bridge species) between two counting sites during one counting session (same season). Each bird species observed in both compartments at the same time was selected as potentially participating in the epidemiological interaction between the two compartments. The estimation of EIs is based on: 1) the assumption that all individuals of the same species belong to the same population which is, given the size of our study site (10km radius around

lake's shores) and the ecology of wild birds, quite reasonable; 2) all individuals in a population have the same health status regarding the HPAI: this cannot be true in practice but given the efficiency of the HPAI virus transmission, it stands as a limitation of this model that will be discussed later.

In the context of a SIR model in multi-host species (Dobson 2004), EIs were estimated using bird counts data and as previously discussed no information about the species-specific susceptibility of wild birds could be incorporated in the model. In infectious disease epidemiology, the effective contact rate (i.e. a contacts that result in the transmission of the pathogen) is the product between the contact rate, the probability of transmission between infected and naive individuals and the pathogen infectious period (Dohoo et al. 2009). The probability of transmission and the infectious period will be assumed constant across species because of the lack of information available and the relative good and homogeneous efficiency of the HPAI to be transmitted between birds. The contact rate between an infected and a susceptible compartment will be dependent on two events (Figure 5.2). The first one is the transmission from an infected compartment to a bridge species and will be dependent on n_i , the number of infected individuals in the infected compartment and the sum of n_b the number of susceptible individuals in each of the bridge species in contact and shared with the susceptible compartment. The second event is the transmission from an infected individual of a bridge species to a susceptible individual of a susceptible compartment and will be dependent of the number of infected individual in the potential bridge species identified (sum of n_b' for each shared bridge species) and the number of susceptible, n_s , of individuals in the susceptible compartment. The probability of transmission will therefore be proportional to the products ($n_i * n_b$) and ($n_b' * n_s$) for each bridge species.

Figure 5.2: Schematic representation of the transmission of a HPAI virus from an infected to a naive bird compartment through bridge species. n_b and n_b' are the sum of the number of observed individuals from each bridge species b in the infected and susceptible compartments respectively; n_i and n_s are the number of individuals from the infected and susceptible compartment respectively exposed to the shared community of bridge species. The spread of a HPAI H5N1 from compartment I to S is equal to the product of probabilities ($P_1 * P_2$), c_1 and c_2 being the contact rates, p the probability of transmission and d the infectious period.



In order to explore on one side the variability of the bridge species component and on the other side the relative exposure of infected and naive compartments to the transmission of the pathogen, the sum of the products ($n_b * n_b'$) later named the Interaction Sum (IS) will be explored first and the variation of n_i and n_s will be discussed later.

The differences between pairs of compartment of the sum of products IS and the species richness of the shared community were tested using a One-way ANOVA (after checking for normality and homogeneity of variance; when heterogeneity of variance was observed, a log transformation was performed): a) between pairs of compartments (across seasons and years), b) between seasons (across pairs of compartments and years) and c) between years (across pairs of compartments and seasons). Differences between pairs of compartments and seasons were tested for both the longitudinal and intensive protocol and difference between years only for the longitudinal protocol.

In order to further explore the composition of the EIs, we use the dominant species (more than 10% of the abundance of the shared community) for each EIs for each counting session for each pair of compartments for the longitudinal protocol and the intensive protocol.

Risk analysis approach

A risk analysis approach as defined by OIE recommendations (OIE 2009) was used to define the risk and the spread pathways in the ecosystem. The standard steps of risk analysis are presented: hazard identification, risk assessment (composed of release, exposure and consequence assessment) and risk management (in discussion section).

Hazard Identification

The hazard is identified as a HPAI strain, which by definition can kill chicken but also potentially other avian species such as ostriches and wild birds.

Framing the risk question

The risk question is defined as the risk of spread of a HPAI strain by bridge species in the four avian compartments once it has been introduced in one of the compartments.

Release assessment

Release assessment concerns the release of the hazard. Here it refers to the release of a HPAI from an infected compartment to a susceptible compartment using bridge species. The release assessment is therefore dependent on n_i , the number of infected individuals in the infected compartment. This number will depend on the host dynamics in each of the compartment. We considered the release possibilities in each of the four compartments by using population dynamics of hosts inferred by questionnaire or ornithological data (Chapter Four - Caron et al. 2011).

Exposure assessment

EIs between compartment are dependent on the number of individuals potentially in contact with bridge species in each compartment n_i (considered in the release assessment) and n_s , the number of naive individuals in the naive compartment, and on the number of individuals n_b and n_b' of each bridge species. Risk exposure was therefore estimated using the IS parameter, as defined above.

Consequence assessment

For each risk estimated a qualitative approach was used by transforming each quantitative or qualitative probability into a three-value classification: low, medium, high. Quantitative probabilities were transformed according to their values and expert assessment. Qualitative probabilities were allocated to one of the three categories using available knowledge and/or expert opinion. The product of probabilities was determined using Table 5.1.

The global risk of spread from one infected compartment to a susceptible compartment was defined as the product of the risk of contact between the infected compartment and bridge species (dependent on n_i), the risk of interaction between compartment (dependent on IS) and the risk of contact between a bridge species and the susceptible compartment (dependent on n_s).



Table 5.1: *Table of correspondence for the products of qualitative probability*

Probability	Low	Medium	High
Low	Low	Low	Low
Medium	Low	Medium	Medium
High	Low	Medium	High

Results

Risk assessment

Release assessment

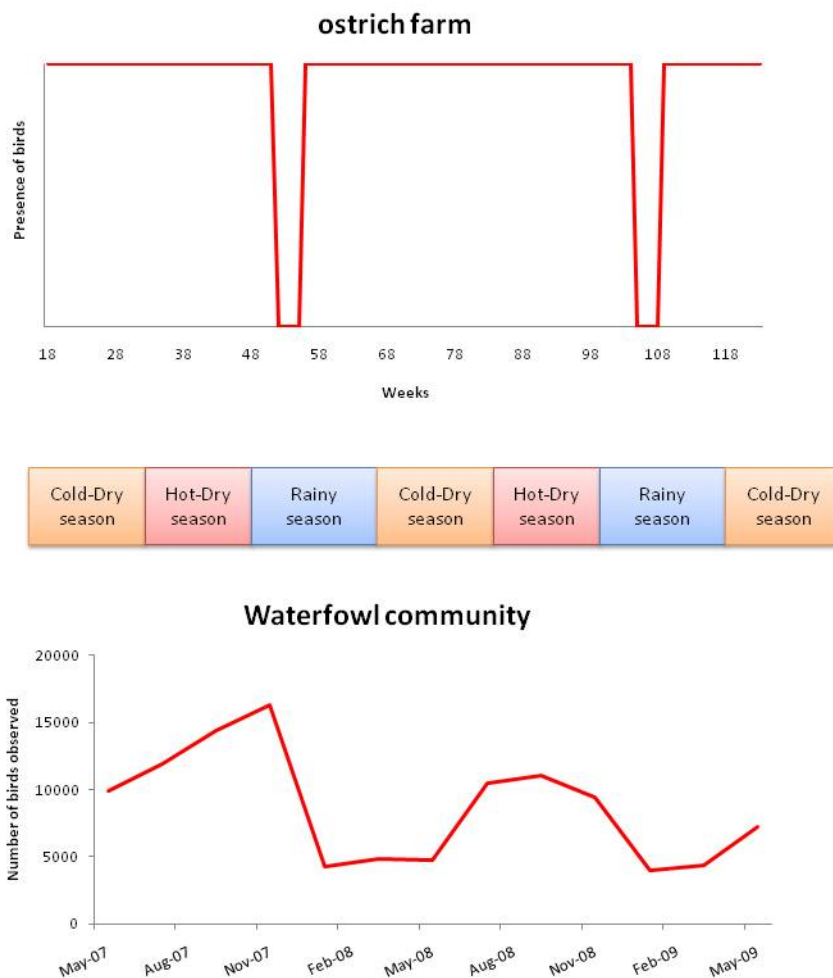
Two possibilities of HPAI release were identified: a) risk of release from the ostrich compartment infected through importation of ostriches or movements of staff; b) risk of release from the waterfowl compartment infected through wild bird movements patterns or local maintenance (Chapter Four - Caron et al. 2011) (Table 5.2). We therefore considered these two entry points for predicting pathogen spread to other compartments in the ecosystem. Importation of ostriches from outside the ecosystem (Bulawayo region, South of Zimbabwe or South Africa) is likely to occur at the beginning of the year when farms are re-stocking meat animals. Therefore ostrich numbers in farms is stable throughout the year from the importation of youngsters in January until their slaughtering in November-December (Figure 5.3). A quarantine period is respected in-between December and January. However staff movements between farms from the Bulawayo area and importation of reproductive birds could in principle take place any time during the year.

In the Waterfowl compartment, based on the emergence risk calculated in Choater Three - Caron et al. (2010), the risk of release of a HPAI strain was higher during the hot-dry season when the highest number of birds was entering the system (mainly Palaearctic and Afro-tropical migrants) (Figure 5.3). However this risk is relative and birds are entering the ecosystem any time during the year.

Table 5.2: Emergence pathways identified in the four avian compartments of the ecosystem

Compartment	Risk of primary emergence in the ecosystem	Justifications
Backyard Poultry	No	<ul style="list-style-type: none"> - No poultry market - Village autoproduction - Village auto-consumption
Intensive Poultry	No	<ul style="list-style-type: none"> - Eggs produced in Harare and delivered at 1 day-old to farms - No exchange between farms - Staff belonging to one farm only
Farmed Ostrich	Yes	<ul style="list-style-type: none"> - Occasional importation of birds from South Africa - Possibility of staff moving from farms outside the ecosystem - Past occurrence of HPAI H5N2 outbreaks
Waterfowl	Yes	<ul style="list-style-type: none"> - Worldwide reservoir of LPAI - Potential spreader of HPAI strains - Regional, continental and international migration of waterfowl

Figure 5.3: Population dynamics in the ostrich and waterfowl compartments on a bi-annual basis (for ostrich, the x axis uses “weeks” as unit when for waterfowl it uses “month”; however, the scale is similar for both graphics). Seasons are represented for a better interpretation of the dynamics. Rainy Season= November to March; Cold-dry season= April to July; Hot-dry season= August to October.



The mean number of ostriches and waterfowl observed in the ostrich and waterfowl compartment respectively during the counting protocols was 189 ostriches and 145 wild birds respectively (in a given area). These numbers reflected the quantity n_i . The number of ostrich in contact with potential bridge species throughout the year was considered constant (as ostrich chicks enter the farm in January and leave the farm for slaughtering 11 months later). The variation of potential n_i from the waterfowl (Figure 5.3) can triple between the rainy season and the end of the dry season. Release from the ostrich compartment was considered as “Medium” when chicks are introduced in the system (January) and “Low” for the rest of the year (based on the fact that young animals are more susceptible to disease compared to adult). Based on the epidemiological data presented in Chapter Four - Caron et al. (2011), risk of release from the waterfowl compartment was considered as: “Medium” from September to November and “Low” for the rest of the year. We concluded that for both emergence pathways, there are higher risk periods and no period during the year when the emergence risk is null.

Exposure assessment

Overall, exposure from the Intensive poultry compartment is difficult to estimate but is the lowest of the four compartments (“Low” risk)(Box 5.1). The Backyard compartment, Waterfowl compartment were both considered as “Medium” risk and the Ostrich farm compartment as “High” risk due to the number of birds exposed.

Box 5.1: *Additional information on the exposure assessment of intensive & backyard chicken, waterfowl and ostriches.*

The mean number of target birds observed during the counting protocol per compartment was 22 chickens, 145 waterfowl, 189 ostriches and 5240 chickens respectively for Backyard, Waterfowl, Ostrich farm and Intensive poultry compartment (counted from the intensive protocol). These numbers reflected the quantity of individual birds n_s , potentially exposed to bridge species for each compartment, except for the intensive poultry compartment. In the Intensive poultry compartment, security measures are put in place to minimise contacts between production chicken and their environment (e.g. confinement in buildings, mesh). However, despite some variability in the level of biosecurity between the seven intensive farms in the study, production chickens roaming outside buildings have been observed in each of them. No systematic recording of this data was made during both protocols but on 19 records of chicken escaped from production building, an average of 12.5 birds per count was observed outside the production buildings (maximum 42 birds). These escapes were the results of holes in the mesh (or size of the mesh in some places not adapted to a few days-old chicks) or staff negligence (gate left open during feeding). No active recovery of the escaped birds was observed and these chickens were observed feeding in proximity to bridge species on several occasions. During sanitary quarantine (with no chicken in the building) between two production cycles in the intensive compartment, bridge species (particularly small passerines) have been observed feeding on food left-over in the production building. This type of behaviour could lead to indirect contacts with bridge species leaving infected material in the building.

- End of the Box -

The mean of IS varied between pair of compartments as presented in Table 5.3 and between years and seasons (Table 5.4). None of the one-way ANOVA tests for differences between compartments, seasons and years for both protocols were significant. However, in the longitudinal protocol, the mean of IS increased from the interface between B/W, I/W and B/I (B, I and W for Backyard poultry, Intensive poultry and Waterfowl compartment respectively). In the intensive protocol, the results were consistent with the longitudinal protocol with $O/W < B/W < I/W < B/O < I/O < B/I$ (O for Ostrich farm compartment).

The species richness varied between compartments for both protocols but varied little between years and seasons. Two one-way ANOVA tests were significant: the difference of species richness between the compartments' interface for both longitudinal ($df=2$, $F=13.1$, $p<0.01$) and intensive protocols ($df=5$, $F=7.4$, $p<0.01$). There were also some consistency in the ranking of the species richness' mean between the two protocols ($I/W < B/I < B/W$).

A high variability of IS was observed intra- and inter-pairs of compartment (Figure 5.4). Dominant species (here more than 25% of IS for a better visualisation) are indicated in Figure 5.4. For the B/I interface, May and November were peak periods almost every year with dominant species being respectively red-billed quelea (*Quelea quelea*) and barn swallow (*Hirunda rustica*). In the B/W interface, no peak season was observed and more species were involved in the dominant species. Barn swallow and cattle egret (*Bubulcus ibis*) were the most common dominant species. Two species of ducks were present as dominant species in two peak periods. In the I/W interface, barn swallow was the dominant species in all four peak counting sessions.

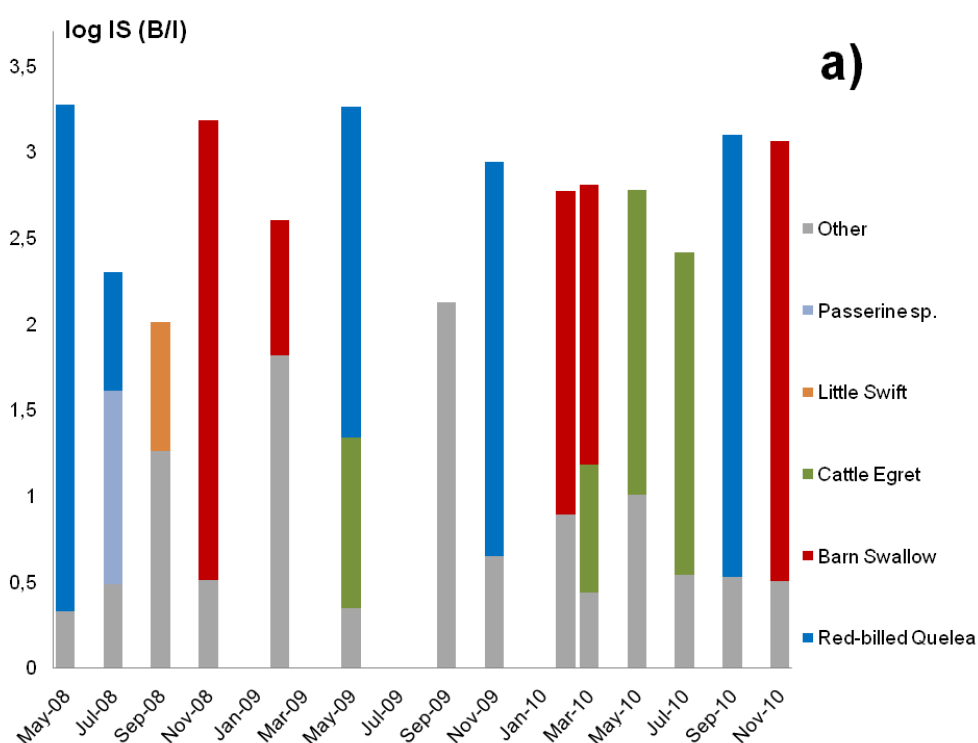
Table 5.3: Means and standard error (Std. Error) of IS (i.e. $\Sigma [n_b * n_b']$) and of species richness (Sp. Rich.) between each pair of compartment (B=Backyard compartment, I=Intensive poultry compartment, W=Waterfowl compartment, O=Ostrich compartment). Each value is calculated by calculating the IS (Mean & Std Error) and species richness indices for each count, for each site for each session. The intensive protocol encompassed 6 sessions, with 15 waterfowl sites, and 6, 6 and 7 sites in respectively backyard poultry, ostrich farm and intensive poultry, with 4 counts per site per session. The longitudinal protocol encompasses 14 sessions, with 7 waterfowl sites, 2 backyard poultry and 2 intensive poultry sites, with 4 counts per site per session.

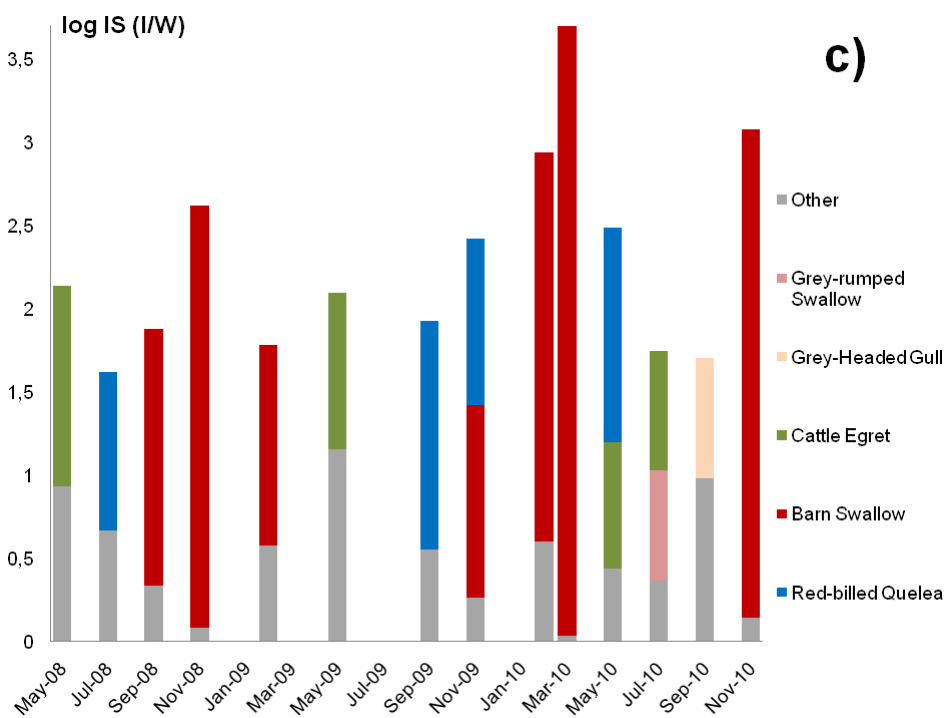
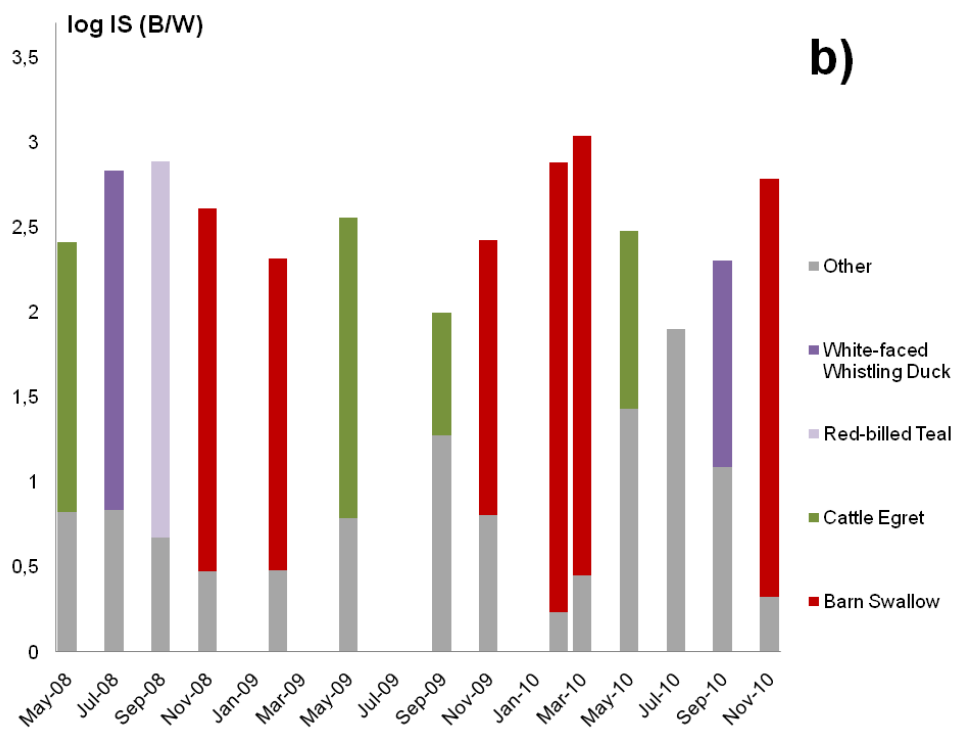
		IS or $\Sigma(n_b * n_b')$		Sp. Rich.	
	Interaction	Mean	Std. Error	Mean	Std. Error
Longitudinal protocol	B/I	819	617	24.6	4.0
	B/W	433	298	28.1	4.2
	I/W	649	1408	20.6	3.4
Intensive protocol	B/I	537	558	53.3	8.3
	B/W	144	74	55.0	9.3
	I/W	212	212	36.0	8.2
	O/W	75	86	63.0	7.6
	I/O	453	560	45.8	7.4
	B/O	334	470	49.8	8.4

Table 5.4: Means and standard error of IS ($\Sigma [n_b * n_b']$), and of species richness (Sp. Rich.) for seasons and years (B=Backyard compartment, I=Intensive poultry compartment, W=Waterfowl compartment, O=Ostrich compartment). The intensive protocol encompassed 6 sessions, with 15 waterfowl sites, and 6, 6 and 7 sites in respectively backyard poultry, ostrich farm and intensive poultry, with 4 counts per site per session. The longitudinal protocol encompasses 14 sessions, with 7 waterfowl sites, 2 backyard poultry and 2 intensive poultry sites, with 4 counts per site per session.

		IS or $\Sigma (nb*nb')$		Sp. Rich.	
		Mean	Std. Error	Mean	Std. Error
Longitudinal protocol	2008	618	655	25.9	6.6
	2009	449	575	26.1	3.6
	2010	632	465	26.1	4.2
	Dry-Cold	475	591	24.0	4.7
	Dry-Hot	527	483	24.9	5.7
	Rainy	1111	1636	24.0	4.0
Intensive protocol	Dry-Cold	408	510	50.8	10.8
	Dry-Hot	222	179	50.2	12.2
	Rainy	87	39	50.3	13.6

Figure 5.4: Variation of the log of the interaction sum (IS) in the longitudinal protocol across the fourteen counting sessions; species representing more than 25% of the interaction sum are indicated; a) Interface between Backyard chicken and Intensive poultry compartments ; b) Interface Backyard chicken and Waterfowl compartments; c) Interface between Intensive poultry and Waterfowl compartments. “Passerine sp.” refers to unidentified small passerines such as red-billed quelea or bronze manikin.





During the longitudinal protocol, all the dominant species (here as >10% of IS) were represented by only 14 species for the three pairs of compartments for the fourteen sessions (eight per pair of compartments) (Table 5.5). Three species were overrepresented: red-billed quelea, barn swallow and cattle egret. Four other species of hirundidae (e.g. swallows, swift and martin) were observed, making this family the most represented bird family in terms of number of species.

During the intensive protocol, all the dominant species for the six pairs of compartments for the 6 sessions encompassing 34 counting sites were represented by only 12 species (Table 5.6). The three same species were also overrepresented (red-billed quelea, barn swallow and cattle egret). In Table 5.5 & 5.6, during high IS period (in grey), the overrepresentation of red-billed quelea, barn swallow and cattle egret was even greater.

In Tables 5.7 & 5.8, qualitative risks already discussed are summarised. In addition, each IS was allocated to “Low”, “Medium” or “High” by dividing each vector of values into three categories. For Table 5.7, classes are: “Low”<150; 150<“Medium”<300; “High”>300. For Table 5.8, classes are: “Low”<210; 210<“Medium”<700; “High”>700.

Table 5.5: *Dominant species for each interaction sum for each session of the longitudinal protocol for each pair of compartments. Dominant species are defined as participating in more than 10% in the total interaction sum. In grey, sessions with highest interaction sum for each pair of compartment. (For B/I, “Passerine sp.” refers to unidentified small passerines such as red-billed quelea or bronze manikin).*

B/I	May-08	Jul-08	Sep-08	Nov-08	Feb-09	May-09	Sep-09	Nov-09	Feb-10	Mar-10	May-10	Jul-10	Sep-10	Nov-10
Red-billed quelea	90%	30%				59%	24%	78%			14%		83%	
Barn Swallow			12%	84%	30%			23%	68%	58%				83%
Cattle Egret					24%	30%				27%	64%	78%		
Bronze Mannikin					21%		18%		11%					
African Palm Swift			20%									12%		
Little Swift			37%				20%							
Passerine Sp.		49%												
Rock Dove							11%							
B/W	May-08	Jul-08	Sep-08	Nov-08	Feb-09	May-09	Sep-09	Nov-09	Feb-10	Mar-10	May-10	Jul-10	Sep-10	Nov-10
Red-billed quelea						17%	20%	24%			18%	15%		
Barn Swallow				82%	79%			67%	92%	85%				88%
Cattle Egret	66%	18%				69%	36%			12%	42%	22%		
Red-billed Teal			77%											
White-faced Whistling Duck	22%	71%	11%										53%	
African Palm Swift												11%		
African Jacana					12%									
Grey Rumped Swallow							17%				32%	30%		
I/W	May-08	Jul-08	Sep-08	Nov-08	Feb-09	May-09	Sep-09	Nov-09	Feb-10	Mar-10	May-10	Jul-10	Sep-10	Nov-10
Red-billed quelea		59%				22%	71%	41%			52%			
Barn Swallow			82%	97%	68%			48%	80%	99%				95%
Cattle Egret	56%	20%			19%	45%			14%		31%	41%		
Bronze Mannikin						20%								
African Palm Swift												14%	16%	
Grey-headed Gull	23%												42%	
Grey Rumped Swallow							10%				12%	38%		
Banded Martin													18%	

Table 5.6: *Dominant species for each interaction sum for each session of the intensive protocol for each pair of compartments. Dominant species are defined as participating in more than 10% in the total interaction sum. In grey, sessions with highest interaction sum for each pair of compartment. (For B/I, “Passerine sp.” refers to unidentified small passerines such as red-billed quelea or bronze manikin).*

B/W	May-08	Jul-08	Sep-08	Nov-08	Jan-09	Mar-09
Red-billed quelea	19%					25%
Barn swallow				55%	39%	
Cattle Egret	44%	25%			32%	55%
White-faced duck	19%	59%	22%			
Red-billed teal			56%			
African jacana					16%	
I/W	May-08	Jul-08	Sep-08	Nov-08	Jan-09	Mar-09
Red-billed quelea	30%	22%	71%	39%		23%
Barn swallow				14%		
Cattle Egret	34%	26%			58%	26%
White-faced duck	11%	29%			14%	18%
Red-billed teal				29%		
O/W	May-08	Jul-08	Sep-08	Nov-08	Jan-09	Mar-09
Red-billed quelea	33%			16%		68%
Barn swallow			64%	80%	44%	
Cattle Egret	28%	39%	12%		50%	11%
Bronze mannikin		13%				
Cape-turtle dove		10%				
B/I	May-08	Jul-08	Sep-08	Nov-08	Jan-09	Mar-09
Red-billed quelea	88%	33%	54%	11%		70%
Barn swallow				26%	16%	
Cattle Egret		20%			25%	18%
Bronze mannikin				31%	20%	
Passerine sp.		23%				
Dark-capped bulbul			10%			
Common waxbill				15%		
Southern red bishop					21%	
B/O	May-08	Jul-08	Sep-08	Nov-08	Jan-09	Mar-09

Red-billed quelea	82%					93%
Barn swallow				68%	60%	
Cattle Egret		14%			17%	
Bronze mannikin		30%	24%	25%		
Cape-turtle dove		35%				
Dark-capped bulbul			21%			
European bee-eater			12%			
I/O	May-08	Jul-08	Sep-08	Nov-08	Jan-09	Mar-09
Red-billed quelea	88%			27%		94%
Barn swallow			13%	45%	24%	
Cattle Egret		13%	13%		51%	
Bronze mannikin		63%	33%	23%		
Dark-capped bulbul			15%			

Table 5.7: Risk Table combining the risk of spread from the ostrich compartment. The risk of interaction is based on the interaction value in the intensive protocol. The Risk of spread is a combination of the three risks presented before (calculation based on -1 for Low, 0 for Medium and +1 for High). In the last column, the main dominant species for the particular session is given.

To Backyard	Risk of introduction	Risk of contact ni/nb=R1	Risk of interaction =R2	Risk of contact nb'/ns=R3	Risk of spread =R1*R2*R3	Dominant species
May-08	Low	High	Medium	Medium	Medium	Red-billed quelea
Jul-08	Low	High	Low	Medium	Low	
Sep-08	Low	High	Low	Medium	Low	
Nov-08	Low	High	High	Medium	Medium	Barn swallow
Jan-09	Medium	High	Low	Medium	Low	
Mar-09	Low	High	High	Medium	Medium	Red-billed quelea
To Intensive	Risk of introduction	Risk of contact ni/nb=R1	Risk of interaction =R2	Risk of contact nb'/ns=R3	Risk of spread =R1*R2*R3	
May-08	Low	High	High	Low	Low	
Jul-08	Low	High	Low	Low	Low	
Sep-08	Low	High	Low	Low	Low	
Nov-08	Low	High	High	Low	Low	
Jan-09	Medium	High	Low	Low	Low	
Mar-09	Low	High	High	Low	Low	
To Waterfowl	Risk of introduction	Risk of contact ni/nb=R1	Risk of interaction =R2	Risk of contact nb'/ns=R3	Risk of spread =R1*R2*R3	
May-08	Low	High	Low	Medium	Low	Barn swallow
Jul-08	Low	High	Low	Medium	Low	
Sep-08	Low	High	Low	Medium	Low	
Nov-08	Low	High	Medium	Medium	Medium	
Jan-09	Medium	High	Low	Medium	Low	
Mar-09	Low	High	Low	Medium	Low	

Table 5.8: Risk Table combining the risk of spread from the waterfowl compartment to the remaining two compartments (the ostrich compartment was not included in the longitudinal protocol) discussed in the text. The risk of interaction is based on the interaction value in the longitudinal protocol. The Risk of spread is a combination of the three risks presented before. In the last column, the main dominant species for the particular session is given.

To Backyard	Risk of introduction	Risk of contact $\Sigma ni / \Sigma nb=R1$	Risk of interaction=R2	Risk of contact $\Sigma nb' / \Sigma ns=R3$	Risk of spread =R1*R2*R3	Dominant species
May-08	Low	Medium	Medium	Medium	Medium	Cattle egret
Jul-08	Low	Medium	High	Medium	Medium	White-faced duck
Sep-08	Medium	Medium	High	Medium	Medium	Red-billed teal
Nov-08	Medium	Medium	Medium	Medium	Medium	Barn swallow
Feb-09	Low	Medium	Low	Medium	Low	
May-09	Low	Medium	Medium	Medium	Medium	Cattle egret
Sep-09	Medium	Medium	Low	Medium	Low	
Nov-09	Medium	Medium	Medium	Medium	Medium	Barn swallow
Feb-10	Low	Medium	High	Medium	Medium	Barn swallow
Mar-10	Low	Medium	High	Medium	Medium	Barn swallow
May-10	Low	Medium	Medium	Medium	Low	
Jul-10	Low	Medium	Low	Medium	Low	
Sep-10	Medium	Medium	Low	Medium	Low	
Nov-10	Medium	Medium	High	Medium	Medium	Barn swallow

To Intensive	Risk of introduction	Risk of contact ni/nb=R1	Risk of interaction=R2	Risk of contact nb'/ns=R3	Risk of spread =R1*R2*R3	Dominant species
May-08	Low	Medium	Low	Low	Low	
Jul-08	Low	Medium	Low	Low	Low	
Sep-08	Medium	Medium	Low	Low	Low	
Nov-08	Medium	Medium	Medium	Low	Low	
Feb-09	Low	Medium	Low	Low	Low	
May-09	Low	Medium	Low	Low	Low	
Sep-09	Medium	Medium	Low	Low	Low	
Nov-09	Medium	Medium	Medium	Low	Low	
Feb-10	Low	Medium	High	Low	Low	
Mar-10	Low	Medium	High	Low	Low	
May-10	Low	Medium	Medium	Low	Low	
Jul-10	Low	Medium	Low	Low	Low	
Sep-10	Medium	Medium	Low	Low	Low	
Nov-10	Medium	Medium	High	Low	Medium	Barn swallow

Discussion

Exposure assessment

The variability of IS and species richness can be summarised as follow: a) there was a (non significant) variability of IS and (significant) variability of species richness between pairs of compartments, indicating that the potential bridge species were not distributed equally between compartments; b) there was a variability of IS between seasons, not significant and not consistent across both protocols but little variability of species diversity; c) there was a (non significant) variability of IS across years but no variability of the species diversity emphasising that the variability of the IS was not only dependent on seasons but also on inter-annual variability.

The variability of the IS and species diversity across pairs of compartment indicated that factors influenced the distribution of potential bridge species between compartments. The habitat surrounding compartments was different in terms of vegetation. Resource availability could attract different species, roosting and reproduction sites also. Additionally, the distance of compartments from each other could also be a confounding factor. For example, all sites were in a radius of 10 kilometres from the lakes' shores, but ostrich farms tended to be on the outskirts of the study sites, while some backyard sites were in direct contact with the lakes' shores. However, the ostrich compartment interface with other compartments did not have a systematically lower IS value compared to other interfaces (Table 5.3). Another factor influencing the difference in IS was the type of farming practices in each compartment which could influence artificial food resource availability: ostriches were fed and watered in pens with open access for potential bridge species. Backyard poultry were not fed in most circumstances and searched for their food. Therefore, the combination of

attractors and environmental variability made the distribution of potential bridge species variable across compartments.

The variability of IS across seasons is a consequence of wild bird ecology (e.g. reproduction, migration and other behavioural adaptation). For example, red-billed queleas are nomadic birds responding to resource availability linked to rainfall. They are known to move in huge numbers (roosting sites of millions of birds; Dallimer and Jones 2002, Hockey et al. 2005). Palaearctic migrants are living Eurasia during autumn and arrive in Zimbabwe in September to depart again around March and April. This seasonality of nomadic and Palaearctic birds is quite evident in Table 5.5 & 5.6 (see red-billed quelea and barn swallow). The variability of IS observed across years can be interpreted by the variability in climatic patterns in the region. Southern Africa is known to have inconsistent and highly variable rainfall patterns (Verschuren et al. 2000). Wild birds respond to this variability through a nomadic behaviour (Dodman and Diagana 2007). As our study focused on one ecosystem, the observed variability can be explained by the differential use of this ecosystem by different wild bird populations in response to rainfall variability.

The lack of variability of species richness across season and years is more complex to explain. The ecological niche concept proposes that one ecosystem can be subdivided into ecological niches for specific host species (Begon et al. 2006). The compartments offered a limited number of niches to bird species and they were constantly occupied during the course of the study. This result could be interesting to explore as the impact of changes in biodiversity on epidemiological processes (given that only an –unknown- fraction of the avian diversity is susceptible to AIV) could be significant. For example, a dilution effect has been proposed for some epidemiological multi-host systems (Keesing et al. 2006).

The result on a smaller dataset that a limited number of species constitutes the majority of IS, was confirmed here with a larger dataset (Chapter Three - Caron et al. 2010). A maximum of four species was above the 10% threshold for all counting sessions per pair of compartment. In the intensive protocol, only twelve species of wild birds were dominant species across the all year of study (6 sessions in 34 sites for 408 hours of counts during one year) and in the longitudinal protocol, thirteen species were found to be dominant in at least one count (out of 14 sessions in 11 sites for 308 hours of counts in three years and half). This number has to be compared with the 249 bird species observed in the waterfowl compartment in the same ecosystem (Chapter Three - Caron et al. 2010). The risk of spread related to bridge species is therefore concentrated in a few bird species. In the 24 counts with the highest IS (out of 78), only barn swallow was involved 14 times, red-billed quelea 12, cattle egret 6 and three other species involved seven times combined (grey column in Table 5.5 & 5.6).

Therefore, we observed much variability in the quantitative estimation of IS with little predictability between compartments, seasons and years. However, we showed that from a qualitative point of view, the IS can be summarised with a few dominant bird species, which, by their ecology, bring some predictability in the interaction between compartments. What are the consequences for the risk pathways identified?

Consequence assessment

The spread of a hypothetical HPAI strain in the ecosystem is considered according to the two introduction routes identified in the release assessment.

- Spread from the ostrich compartment (intensive protocol)

Overall, the risk of spread from the ostrich compartment is low (Table 5.7). The risk of spread to the Backyard compartment is higher than to the Waterfowl compartment and both are higher than the risk of spread to the Intensive compartment. The risk of diffusion from the Ostrich compartment is low when the highest risk of introduction in this compartment is the highest (January) and is Medium a couple of month later in March and May when red-billed queleas are in the ecosystem and in November when barn swallows are present in numbers. The risk of spread from the Ostrich compartment is therefore higher when red-billed queleas and barn swallows are in the ecosystem. For the former, the March and May period is therefore at risk, for the later mainly September-November. Inter-annual variability can shift these periods.

- Spread from the Waterfowl compartment (longitudinal protocol)

The overall risk of diffusion from the Waterfowl compartment was Low towards the Intensive compartment and Medium towards the Backyard compartment (Table 5.8). The high risk period occurred during the dry-hot season and to a lesser extent in February-March, just before Palaearctic birds such as barn swallows left the ecosystem. In May 2008 and 2009, the risk of spread from the Waterfowl to the Backyard compartments was considered as Medium and cattle egret were the dominant species for each period. In May 2010, cattle egret was again the dominant species although the risk of spread was from the Waterfowl to the Backyard compartments considered Low. The fact that the peak season for IS coincided with the highest risk for HPAI strain introduction in the Waterfowl compartment increased the risk of diffusion of the pathogenic strain the ecosystem.

Risk Management

The decision to use this level of analysis instead of finer estimators of contact such as individual telemetry or direct observation of contacts was based on its repeatability using cost-effective counting protocols or already available ornithological databases. The risk estimation presented here is not absolute. In practice, parameters of the transmission event assumed to be constant in Figure 5.2 are not. First, the prevalence in the infected compartments is not constant across species. The definition of a HPAI strain is based on the mortality induced in poultry chicks (Alexander 2000). This does not mean that this strain produces mortality in ostrich or in waterfowl. The contrary has been shown in various studies on the pathogenicity of HPAI strain in ostriches (Capua et al. 2000). This variability in pathogenicity can have consequences on the number of birds infected in the compartment and the time lag between introduction and detection (if birds do not die, the outbreak could go undetected). We focus on the contact rate between hosts, but not all contacts between hosts are followed by the transmission of the pathogen (McCallum et al. 2001), and some contacts could have a higher probability of pathogen transmission compared to others, adding heterogeneity in the model. The susceptibility of the bird in contact with an infected individual is highly variable and dependent on its personal history (immunity) and on the susceptibility of the species to the particular pathogen and strain (some host species may be more susceptible to some strains than others). However, this study had the objective to investigate the potential bridge species dynamics between compartments, seasons and years. Its results have implication for disease management. There are other transmission pathways for a HPAI strain in this ecosystem, but they have not been addressed here (e.g. human movements between compartments).

There has been so far little work on bridge species and the reason could be that the complexity of this multi-host system was not possible to address. Our findings suggest that in

this Zimbabwean ecosystem, there are only a few key species visiting the ecosystem at specific time of the year which constitutes the majority of the epidemiological interactions between compartments. Furthermore, the risk of spread varies in relation to bird species ecology (e.g. annual palearctic migration of barn swallows, nomadic movements of red-billed queleas, reproductive behaviour of cattle egret). This has direct implication for the management of the HPAI risk if it was introduced in this ecosystem. Management options to limit contact between production stock and these key species during high risk seasons are possible. For example, red-billed quelea is a pest in Southern Africa and a variety of control options exist to avoid their feeding on crops. Barn swallows are mainly visiting production unit to feed on insects. Insect control could reduce these visits. Modification of the habitat could also reduce roosting sites at proximity of production buildings. Cattle egrets are usually following cattle in the proximity of farms. Avoiding cattle visiting those farms would reduce the interface. A few control options could therefore drastically reduce the risk of spread of the pathogen between compartments. However the inter-annual variability observed would require a monitoring system to make sure the control options are put in place when the risk is present. In 2010, our field team looked for red-billed quelea roosting sites around the lakes as they have been observed at this season for the past two years. The main roosting colonies arrived only in the ecosystem in September 2010. This change in behaviour could be an adaptation to the late rains which occurred that year in April. As can be seen in Figure 5.4, the red-billed quelea risk has shifted from May to September-November this year.

The protocol presented here is time consuming but cost-effective. Instead of sampling blindly the avian community, our protocol structures the approach and targets the bird species with the highest impact on disease transmission. We can present to level of validation of our model; First, barn swallows, red-billed queleas and cattle egrets have been shown to be susceptible to AIV in other ecosystems (Gronesova et al. 2008, Mizakova et al. 2008, Squires

et al. 2008, Breithaupt et al. 2010) indicating that they should be susceptible also in our ecosystem. Second, each of these potential bridge species has been sampled during high risk period highlighted by this model: barn swallows and red-billed queleas have been found to be positive to AIV by RT-PCR technique when cattle egrets have not. Therefore, if one of these species is infected by a HPAI strain, the risk of its diffusion in the ecosystem would be possible and controllable. As presented in Chapter Four - Caron et al. (2011), the Waterfowl compartment is hosting LPAI strain all year long. In the three domestic compartments, positives RT-PCR detection of LPAI strain were found (Caron, unpublished data). Therefore the risk of a HPAI diffusing in this ecosystem represents a reality and the bridge species pathways a good candidate to explain these results.

From a more theoretical point of view, it would be interesting to investigate bridge species communities in other ecosystems to assess if our findings are site specific or not. If not, in addition to offer a framework to identify potential bridge species at the ecosystem level, our approach could help exploring potential rules of bridge species communities between avian compartments. If similar findings are found elsewhere, this would mean that the risk of spread of pathogens by bridge species in an ecosystem is the result of the epidemiological interaction of a few key species, offering a limited number of control options with potential high impact on the sanitary risk.

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Chapter Six: Exploring the relation between avian communities and AIV ecology in Southern Africa using the concept of epidemiological functional groups

(Chapter reference: Caron, A., de Garine-Wichatitsky, M., Ndlovu, M., Cumming, G. S. (In Prep) Exploring the relation between avian communities and AIV ecology in Southern Africa using the epidemiological functional group concept)



Introduction

The ecology of pathogens and the emergence of disease in multi-host systems is complex (Woolhouse et al. 2001, Haydon et al. 2002), and understanding it often requires the incorporation of a wide variety of different kinds of evidence and different disciplinary approaches (Plowright et al. 2008). Traditional surveillance and control approaches have often focused on humans, domestic animals, and known vectors. However, an increasing body of information indicates that effective disease surveillance and control may be heavily dependent on understanding the epidemiology of pathogens in wild hosts and the ecology of these hosts (e.g. Chevalier et al. 2004, Olsen et al. 2006, Woodroffe et al. 2008, Leroy et al. 2009).

As more hosts are considered in an epidemiological system, understanding the specific relationship between each host and the pathogen (e.g. susceptibility, pathogenicity) in order to assign each hosts to a specific role in the epidemiological cycle (e.g. reservoir, dead end-host, spreader) quickly becomes challenging. There is therefore a need to summarize this complexity without oversimplifying it. A good starting model for system simplification comes from the field of community ecology, in which researchers have attempted for some decades to deconstruct the complexity of food webs (May 2006). Concepts such as trophic levels and foraging guilds have played an important role in the development of ecological theory, and successful approaches should in theory be readily modifiable to facilitate the analysis of the ecology of pathogen transmission in multi-host systems. The concept of epidemiological functional groups (EFGs) uses epidemiological roles instead of foraging guilds to classify hosts into groups that capture their role in the epidemiology of a pathogen or a group of pathogens (Chapter Seven - Caron et al. Submitted). Hosts in a group share a common function in the epidemiology of the pathogen(s) of interest. In this study we further develop

the concept of EFGs by using them as a lens through which to investigate the ecology of avian influenza viruses (AIV) in wild avian communities in Southern Africa.

AIVs in wild birds have recently received much attention due to the Highly Pathogenic AIV H5N1 strain epizooty and its potential threat to human health (Capua and Alexander 2002). Although numerous studies of low pathogenic AIV strains (LPAI) in waterfowl and wild birds have been published, encompassing tens of thousands of sampled wild birds, there is still little information on the susceptibility of individual bird species to AIV in relation to the global number of bird species (Olsen et al. 2006). Avian communities in a given ecosystem can span hundreds of interacting species. The use of EFGs in this context, based on known or hypothesised facts, should provide a powerful tool for exploring global patterns of AIV ecology. Most studies of AIV have concentrated on Anseriformes and Charadriiformes, which are known to be reservoirs for LPAI (Webster et al. 1992, Olsen et al. 2006). Little information on AI prevalence in the rest of the avian community has been published, and much of what has been published has been obtained as “by-catch” of capture protocols that have been focused on ducks. The minimum sample sizes that would be necessary to confidently estimate prevalence for most non-target bird species are thus often not reached.

In addition, lack of information regarding the composition of the wild bird community from which the sample is taken makes conclusions from AIV studies difficult to interpret. A total of 100 positive samples from species A, for example, carries a different epidemiological weight if species A represents 0.1% versus 90% of the number of wild birds present in the ecosystem; and similarly, the relevance of 100 positive samples from one species differs if the system contains 10 or 100 other species. Interpretation of the role for pathogen maintenance of species A cannot be made rigorously without considering the potential role of the rest of the community. Experimental infection trials indicate that it is impossible to predict the

susceptibility of species according to its ecology or phylogeny (Ellis et al. 2004, Werner et al. 2007, Brown et al. 2009). Determining the role of a particular species in AIV ecology is therefore heavily contingent on direct experimental or field results. In this article we use two years of regular and consistent bird census and epidemiological data on AIV in wild birds to explore AIV epidemiology in the context of the avian community in three different ecosystems across Southern Africa.

Our analysis followed three main steps: (1) comparison of the waterfowl communities' characteristics across the 3 sites; (2) comparison of the representativity of the epidemiological data for each site; and (3), bringing these two strands together, analysis of two Epidemiological Functional Groups that are based on known characteristics of AIV ecology in wild birds. The first ecological function (EF) relates to maintenance and non-maintenance functional groups (Figure 6.1). The target population (according to (Haydon et al. 2002) definition) is at risk of AIV transmission from the maintenance population directly or indirectly through the non-maintenance population. The second EF concerns the patterns of movements of wild bird species relative to the ecosystem (Figure 6.2). As birds move or migrate further from a given ecosystem, they will be exposed to more diverse AIV strains and could introduce those strains in the ecosystem. Depending on the circulation of AIV in the ecosystem under study, the introduction of exogenous strains could trigger epizooties if no immunity against this strain exists. These introductions could also play a role in the reassortment processes and the emergence of new strains (Webster and Hulse 2004, Chapter Two - Caron et al. 2009).

Figure 6.1: *Epidemiological functional group 1: maintenance and non-maintenance community in relation to target population (here the domestic bird population). The maintenance community host the virus and maintain it. The non-maintenance community can transmit the viruses to the target population but cannot maintain it.*

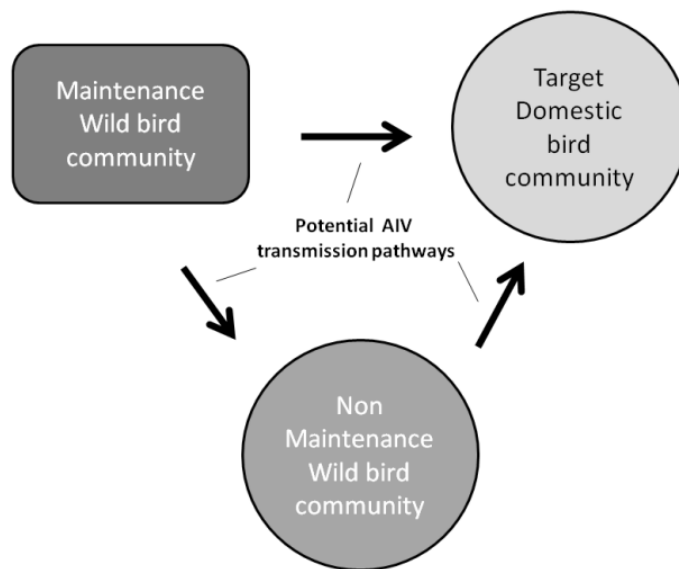
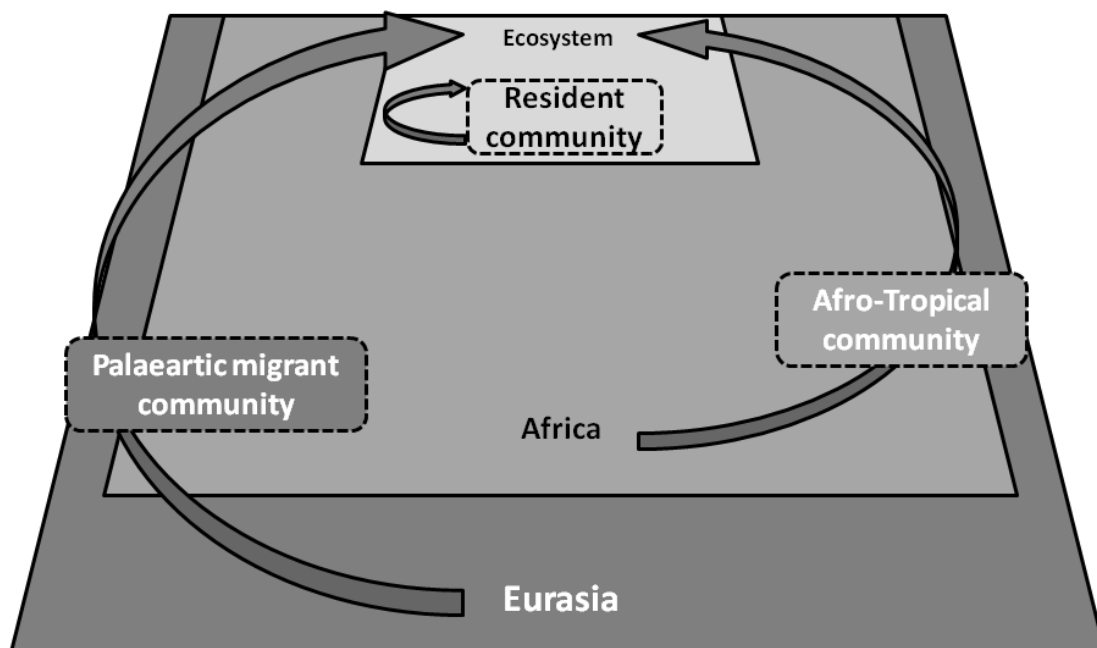


Figure 6.2: *Epidemiological Functional Group 2: bird species are allocated to groups according to their movement behaviour. Resident species do not leave the ecosystem; Afro-tropical species move within Southern Africa and/or African regions on both sides of the equator; Palaearctic migrants move seasonally between Eurasia and Africa. Arrows represent the potential for AIV strain introduction in the focal ecosystem from different origins for each EFG.*



Methods

Study sites

Three sites in Southern Africa were investigated: (1) Barberspan (BAR) in Gauteng province, South Africa, is a RAMSAR wetland of total area varying between 1000 and 1700 ha; (2) Strandfontein wastewater treatment works (STR) in the Western Cape, South Africa, is a 319 ha water body located in Muizenberg on the immediate periphery of the city of Cape Town; and (3) the Manyame-Chivero dams (MAN) in Zimbabwe, which are man-made impoundments that are linked by the Manyame river and were built in the 1950s to supply the city of Harare with water. They cover areas of 6500 and 18500 ha respectively. More information on these sites is available as supplementary material in Appendix Five - Cumming et al. (2011).

Baseline data

Bird census data was collected using point counts for two years in each site from February 2007 to May 2009. Each point count consisted of a 10-minute habituation period followed by a 30 minute focal count of all birds in a semi-circle of 150m radius, facing the waterbody. Point counts were undertaken at 12 to 15 points at each of our three sites (BAR, STR, and MAN) and were repeated four times at each location over five days during each counting and sampling session. Sessions were repeated every two months.

AIV prevalence was estimated by sampling captured birds every two months during two years in each site from February 2007 to March 2009. The capture sessions were undertaken during a week immediately following each 5-day counting session. Walk-in traps and mist nets were used to capture wild birds on the water body shores, with occasional use of

spring- or canon-nets. Additional details on the protocols have been given in Appendix Five - Cumming et al. (2011).

Data analysis

First step: comparison of bird communities between sites

Four complementary metrics were calculated to describe the waterfowl community in the three sites: species richness (total number of species), Shannon's diversity index (Shannon and Weaver 1949), species evenness, and the concentration ratio (proportion of the most represented species; e.g. Concentration Ratio 4 gives the proportions of the four most represented species). All metrics were calculated across the two years of counting. The bird species of the 3 sites were allocated to EFGs using available regional knowledge (Hockey et al. 2005) and the composition of these groups was compared across ecosystems (see below).

Second step: comparison of birds sampled and birds counted

We estimated the bias in terms of waterfowl community representativity in the sample induced by the bird capture techniques and the "catchability" of waterfowl species by comparing the proportion of each bird group captured and observed across the two years of capture.

Third step: prevalence & EFGs

The possibility of an endemic cycle has been raised by previous studies in Southern Africa (Chapter Four – Caron et al. 2011, Appendix Five - Cumming et al. 2011). The allocation of bird species into EFs is made on the basis of available knowledge and when no information is available for a set of species, they are grouped together by default. This approach allows the exploration of the possible relevance for AIV dynamics of a group of

species for which so little information is available that we are unable to say anything definite about their role in AIV epidemiology.

Epidemiological function 1, EF1, is related to the known and unknown role of bird orders in the maintenance of AIV in an ecosystem. Anseriformes and Charadriiformes are bird orders considered globally as reservoirs for AIV and many studies consider only these two orders for epidemiological investigations (e.g. Ito et al. 1995, Hansbro 2010). If there is an endemic AIV cycle in Southern Africa, we hypothesized that Anseriformes (and potentially Charadriiformes) would constitute the maintenance community. We allocated Anseriformes and Charadriiformes into two different maintenance EFGs because they do not always share the same viral pool and do not always share transmission pathways (Olsen et al. 2006). In Africa, a role as a reservoir for both groups has been suggested by recent studies (Appendix One - Gaidet et al. 2007, Chapter Four - Caron et al. 2011, Appendix Five - Cumming et al. 2011, Appendix Four - Gaidet et al. 2011). The other bird orders have not been investigated enough to allocate different groups to different roles in viral maintenance. We have therefore defined the three following groups: *Ans* (Anseriformes), *Cha* (Charadriiformes) and *RoC* group (Rest of Community), the later regrouping all non Anseriformes and non Charadriiformes bird species. If Anseriformes and Charadriiformes represent the main reservoir of AIV in Southern Africa, the *RoC* group should play a minor role in the ecology of AIV with occasional spillover of AIV strains triggering infections; and the estimated prevalence in this group should be lower than in the two other groups across the two years of study.

EF2 focuses on the capacity of a bird species to introduce AIV strains from different ecosystems across regions or continents. Southern Africa has never experienced any HPAI H5N1 outbreak but experiences recurrent outbreaks of HPAI H5N2 in ostriches (Sinclair et al. 2009, OIE 2011). It is therefore important when considering epidemiological functions to

differentiate bird species according to their movement patterns. We allocated birds in our study communities to the following groups: a) Long range spreader or Palearctic (*Pal*) migrant, migrating from Eurasia where high prevalence of AIV including HPAI strains occurs (Wallensten et al. 2007); b) Middle range spreader or Afrotropical migrant, migrating North of the equator in Africa where HPAI H5N1 has become endemic in some regions; c) Small-scale spreader or nomad, moving regionally to follow resources and/or undertake moult or breeding-related local migrations; and d) Non spreader or Resident (*Res*) bird with limited local movements.

Despite the availability of detailed information about wild bird in Southern Africa (Hockey et al. 2005), the behaviour of some species remains unclear, particularly where two or more populations of the same species can behave differently. We therefore decided to regroup medium and local-scale spreader species into a single *Afr* (mobile Afro-tropical) group. A role for Palearctic birds in the introduction of Eurasian AIV strain in Africa has been suggested (Abolnik et al. 2006, Cattoli et al. 2009). If there is no endemicity of AIV in Africa, we hypothesized that Palearctic migrants should introduce AIV regularly in these ecosystems. By contrast, a community dominated by the “Resident” EFG should experience little AIV circulation. For each EF and for each site, we calculated the prevalence of AIV across all members of each EFG.

Results

First step: comparison of waterfowl communities between sites

Comparing diversity indices across the three study sites (Table 6.1), MAN has higher species richness, a higher evenness and therefore a higher Shannon index than STR and BAR.

By contrast, the bird density (represented by the total number of birds observed divided by the total number of counts, given that all counts were undertaken within a 150m semicircle) in MAN is inferior to the bird densities in STR & BAR. The concentration ratio (CR) at four and eight species was also inferior to the CRs in STR and BAR. Differences between BAR and STR were smaller: STR is less diverse (139 against 199 species recorded) and the values of the Shannon and evenness indices were smaller in BAR. Concentration ratios for the three sites were quite high, meaning that the first 4 and 8 species represented a high proportion of the global community.

The community composition relative to EF1 and EF2 across the three sites differed (Table 6.1). BAR and MAN were dominated by the *RoC* group. STR had a higher proportion of *Cha*, slightly higher than *RoC* (40.8% compared to 39.7%). Densities of *Ans* were similar across the three sites but their proportion was higher in MAN compared to the two other sites. *Afr* dominated all three bird communities. For proportions of *Res* species, MAN > STR > BAR. *Pal* species represented a higher proportion in MAN compared to BAR then to STR. When combining EFG 1 and 2 (Figure 6.3), the community composition varied even more between the three communities. BAR & STR were dominated by *RoC-Afr*, whereas MAN was dominated by *Ans-Afr*. STR had more *Cha-Res* compared to BAR & MAN. Densities were smaller in MAN compared to STR & BAR (as already mentioned for Table 6.1).

Second step: comparison of bird sampled and bird observed

The proportions of bird groups observed and sampled differed between sites (Figure 6.3). In all three sites the Anseriformes family was overrepresented in the sampled birds, primarily reflecting the use of specific capture techniques (e.g. walk-in traps) to target this bird family. In addition, some observed dominant bird families at the community level are

poorly represented or absent from the samples. This was the case for the *RoC-Afr* group for all three sites and for *Char-Afr* in STR. No Palaearctic waders were sampled at STR.

Third step: Prevalence & EFGs

Ans-Afr represented the only Anseriformes present in the three sites and their AIV prevalence was 1.1, 1.2, and 5.0% respectively for BAR, STR & MAN (Figure 6.4). *Cha* in the three categories of EF2 had zero prevalence at both BAR and STR, albeit with small sample sizes. At MAN, *Char-Afr* had a relatively high AIV prevalence (as for *Char-Pal*) but with a large confidence interval. The *RoC* group has non-zero prevalence in the three sites for *Res* and *Afr* for BAR, *Afr* for STR and all three groups for MAN. Bars in the background of Figure 6.4 represent the proportion of birds observed for each group. Except for *Cha-Afr* (but only 38 individuals sampled), any bird groups representing more than 15% of the community had a non-zero AIV prevalence.



Table 6.1: Indicators of waterfowl community diversity: "Birds Obs/Count": average number of birds observed per count and standard error displayed; "Species richness": number of species observed across the two years; "Shannon's index" & "Evenness": both diversity index; "CR4" & "CR8" concentration ratios of the first four and eight species respectively. Proportion of each combined groups of EF 1 & 2 are displayed in each ecosystem (Ans=Anseriformes, Cha=Charadriiformes, RoC=Rest of Community, Res=Resident, Afr=Afro-tropical, Pal=Palearctic).

	BAR	STR	MAN
Bird Obs/Count	246±537	234±216	144±171
Species richness	198	138	249
Shannon's index	2.72	2.95	3.54
Evenness	0.514	0.598	0.641
CR4	64.9%	53.3%	43.6%
CR8	76.1%	70.7%	56.7%
Ans	17.0%	19.5%	34.0%
Char	10.8%	40.8%	22.6%
RoC	72.2%	39.7%	43.4%
Res	7.0%	12.7%	14.5%
Afr	88.4%	84.0%	78.4%
Pal	4.6%	3.3%	7.1%

Figure 6.3: Community observed (left) and captured (right) in the three sites according to EF 1 & 2 groups. Bird density (“Observed”) is calculated by the number of birds observed divided by the number of counts (counts implemented in a given area). Bird abundance (“Captured”) is the number of birds captured. Dark grey = Anseriformes, Medium grey=RoC and Light Grey=Charadriiformes.

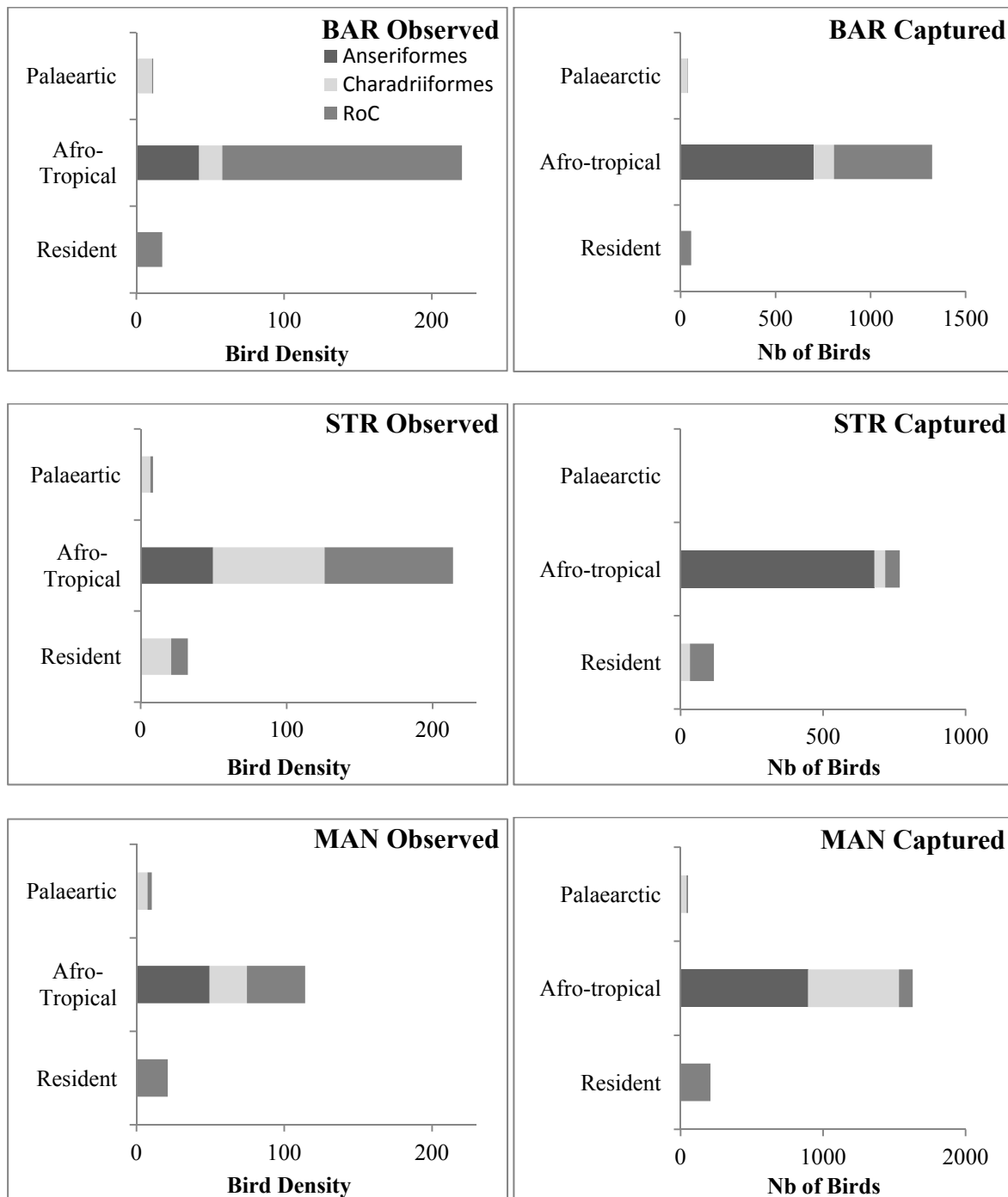
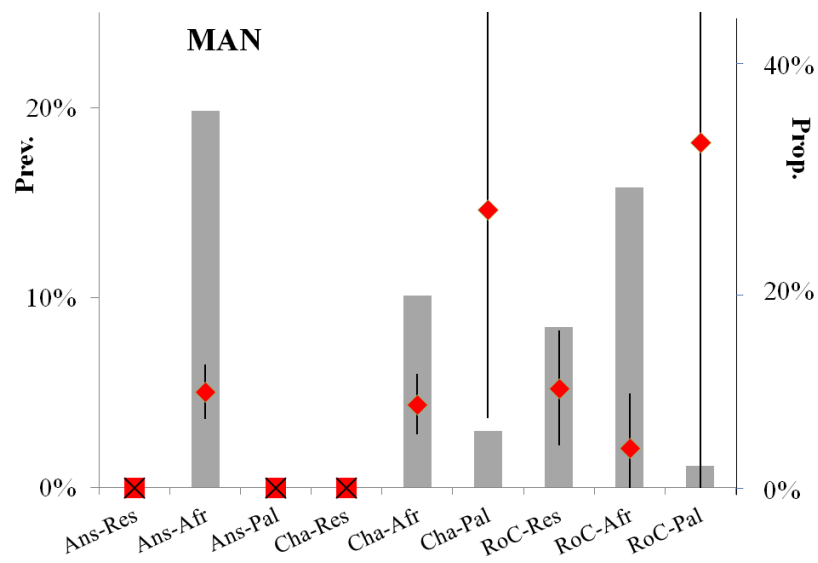
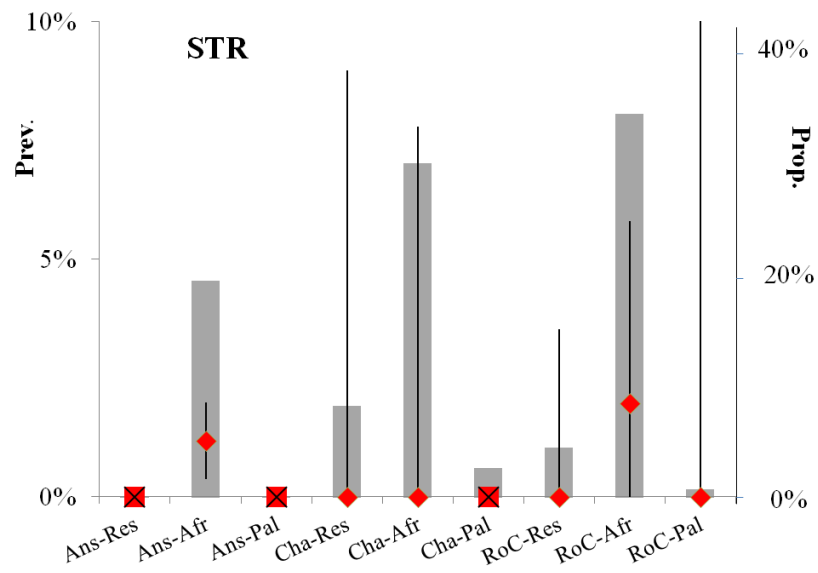
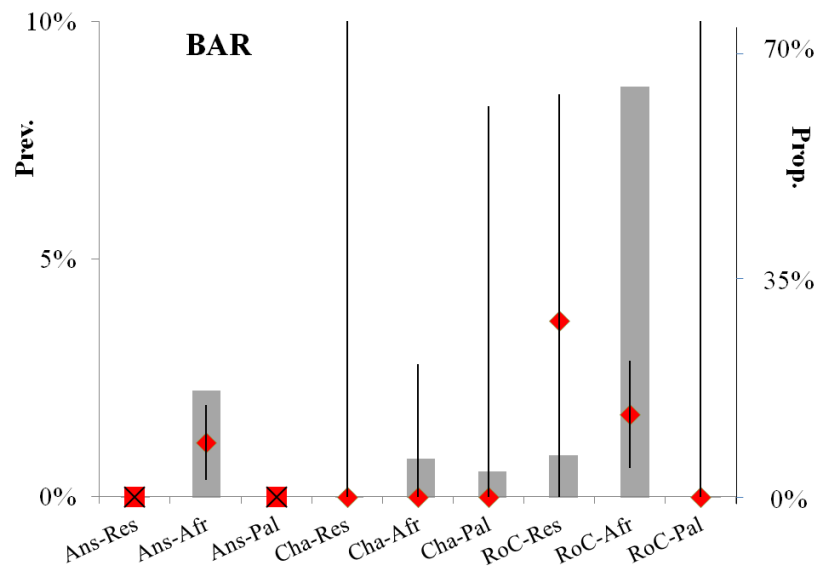


Table 6.2: Prevalence (Prev) of AIV and confidence interval (CI, Lower and Upper boundaries) at 95% calculated for each site across the 12 sampling sessions at the community level and for each group of both EFs. *n* = number of birds sampled; Prev. = Estimated Prevalence (based on results presented in Appendix Five - Cumming et al. (2011)).

	BAR				STR				MAN			
	n	Prev (%)	CI (%) Lower	Upper	n	Prev (%)	CI (%) Lower	Upper	n	Prev (%)	CI (%) Lower	Upper
Global Community	1418	1.3	0.7	1.9	887	1.0	0.4	1.7	1891	5.0	4.0	5.9
Ans-Res	0	na	na	na	0	na	na	na	0	na	na	na
Ans-Afr	701	1.1	0.4	1.9	680	1.2	0.4	2.0	894	5.0	3.6	6.5
Ans-Pal	0	na	na	na	0	na	na	na	0	na	na	na
Cha-Res	2	0.0	0.0	98.0	33	0.0	0.0	9.0	0	na	na	na
Cha-Afr	106	0.0	0.0	2.8	38	0.0	0.0	7.8	639	4.4	2.8	6.0
Cha-Pal	36	0.0	0.0	8.2	0	na	na	na	41	14.6	3.7	25.6
RoC-Res	54	3.7	0.0	8.5	84	0.0	0.0	3.5	210	5.2	2.2	8.3
RoC-Afr	517	1.7	0.6	2.9	51	2.0	0.0	5.8	96	2.1	0.0	5.0
RoC-Pal	2	0.0	0.0	98.0	1	0.0	0.0	100.0	11	18.2	0.0	42.1

Figure 6.4: For each site (BAR, STR, MAN): a) prevalence and confidence interval (left axis) for each combination between EF1 & EF2 (Ans=Anseriformes, Cha=Charadriiformes, RoC=Rest of Community, Res=Resident, Afr =Afro-tropical, Pal=Palearctic migrant); b) Proportion of each bird group in the bird community observed (or counted) during the 2 years of the project (right axis).



Discussion

The data that are needed to compare the estimated AIV prevalence from a non-random host sample with the global host community available across a two-year period have not previously been assembled. *A priori*, we expected to find that different host community compositions in different ecosystems should lead to different epidemiological patterns.

First step: comparison of bird communities between sites

The bird communities differ between the three sites. MAN differs from BAR and STR in almost every index of community (Table 6.1). To summarize, MAN is more diverse, more even in terms of species composition and bird density is lower compared to BAR and STR. The larger size of the MAN ecosystem compared to the two other wetlands could explain this difference in density. It is important to note that if MAN, STR and BAR do not differ in *Ans-Afr* density, they differ in the proportion in the total community (Figure 6.3). Based on available information about AIV ecology in waterfowl, the community composition in MAN is more favourable to AIV maintenance because the community is dominated by Anseriformes. In STR, the important presence of Charadriiformes suggests the possibility of AIV maintenance. In BAR, the *RoC* group dominates: as little information is available on the numerous species composing this group, inferences on AIV circulation are difficult to make.

There are no *Ans-Pal* reaching Southern Africa and only a few Anseriformes belonging to the *RoC* group. *Pal* are present in small proportion in all three ecosystems, most of them being Charadriiformes. There is potential for AIV introduction through seasonal movements of these *Pal species*, when they arrive in the region from Eurasia, in late September-early October. Most birds in the three communities are *Afr* (88.4, 84.0 and 78.4% for respectively BAR, STR and MAN). This *Afr* group encompasses African migratory and nomadic species using local resource availability as a driver for regional movements

(Dodman and Diagona 2007). For some species in this group, it is not known what proportion of the population undertake nomadic movements and trans-equatorial migration (e.g. red-billed teal, *Anas erythroryncha*). These gaps in knowledge prevent the separation of the *Afr* group into two.

The representativity of the bird communities observed in each site of this study is not perfect: focal counts from the shore line of water bodies cannot census all bird species. The role of water bodies considered in relation to other water bodies in the direct vicinity (non-perennial and perennial) is also important to consider. Birds moving from one water body to the next can spread pathogens and resource availability attracting birds at different seasons can create a meta-population system for AIV with extinction and introduction events in the local network of water bodies. Sampling in a single water body will therefore be biased by the role of this water body in a broader network. However, in the three ecosystem studied, the water bodies sampled constitute the main ones in the vicinity.

Second step: comparison bird sampled and bird observed

Sampling in wild populations is biased in several ways (Morgan et al. 2004, Yasue et al. 2006). As for many studies investigating the relation between wild birds and AIV, this study initially aimed at waterfowl and in particular Anseriformes as no information was available about AIV ecology in these ecosystems. Our initial objective was to test the most common hypothesis of Anseriformes as the main reservoir for AIV in waterfowl. Secondly, the bias observed is also a consequence of the catchability of wild birds in general. Most Anseriformes are easy to catch using baited walk-in traps. Charadriiformes can be difficult to catch as you need expertise to set mist nets at the appropriate location and time of the day. Our sampling composition reflects these issues and highlights the bias in prevalence that can be introduced by waterfowl sampling. All the non-target species captured as by-catch have

been sampled. As a result the representativity of the sample size in relation to the group composition is not well respected as on Figure 4, in STR, the *Cha-Afr* & *Roc-Afr* groups. The juxtaposition of community composition and sampling allow identifying future groups to be targeted in this community in order to complete the epidemiological picture.

Third step: AIV prevalence estimation using the EFG approach

The EFG approach considers groups of avian hosts according to their functional role relative to AIV epidemiology. Such approach decreases the complexity of multi-host systems but includes inevitable approximations. In EF1, if the first two groups, *Ans* and *Cha* are well defined taxonomic groups, the third group, *RoC*, brings together more than a hundred species for each site with little information about their respective role in AIV epidemiology. Similarly in EF2, the grouping of bird species according to movement patterns is approximate. The complexity and flexibility of animal behaviour lead some species to behave differently according to the population they belong to and to their environment. The nomadic behaviour of many species in Southern Africa complicates the picture as bird movements are driven by local patterns of rainfall known to be unpredictable from one year to the next (Dodman and Diagana 2007).

Despite a similar *Ans-Afr* density in the three sites (Figure 6.1), the AIV estimated prevalence differs significantly between MAN (5.0%) and BAR (1.1%) and STR (1.2%) (Figure 6.4, both chi-square tests being highly significant, $p < 0.001$). This observation can be explained by two hypotheses: a) as MAN is a much larger area than BAR and STR, the total *Ans-Afr* population is a better predictor of AIV prevalence, compared to their density; b) the composition of the rest of the host community has an influence on the level of AIV circulation in Anseriformes. The second hypothesis is supported by the estimated prevalence in the other EFGs. All groups in MAN have a non-zero mean prevalence. The MAN prevalence for *RoC*-

Res and *RoC-Afr* are significantly higher than BAR *RoC-Afr* (chi-square test, $p < 0.01$ and $p < 0.05$ respectively) and higher but not significantly (because the sample size for the two following groups is small) from BAR *RoC-Res* and STR *RoC-Afr*. The prebalance of *RoC* and *Cha* groups in MAN are not significantly different from the *Ans-Afr* group. In BAR and STR, the overall AIV prevalence is lower than in MAN and seems to be relatively similar in well-sampled groups. These observations cannot prove which of the above hypotheses are relevant but they support a role of the non-Anseriformes groups in the AIV prevalence in these ecosystems. The temporal examination of this data led to the same interpretation between duck and non-duck species (Chapter Four - Caron et al. 2011).

In terms of number of AIV infected birds in each of the three sites (by multiplying prevalence by community proportion in Figure 6.4), in BAR and STR, the number of *Ans-Afr* infected is lower than *RoC-Afr* in both sites. In MAN, *Ans-Afr* would represent the group with the highest number of infected birds but the sum of the 5 other groups with an estimated prevalence would be higher. Therefore, in the three sites, our results indicate that there are more non-Anseriformes infected birds than Anseriformes infected birds. These results point again at a role played by non-Anseriformes groups in the maintenance of AIV in these ecosystems (Stallknecht and Brown 2007). The *RoC* groups represent more than 100 species. Most of the species in these groups had zero positive individuals for a small sample size. However a few others are driving the prevalence at the group level and proper sampling should be implemented for these species in order to clarify their role. For some terrestrial species, experimental data suggests a potential role in virus shedding (e.g. Breithaupt et al. 2010, Forrest et al. 2010, Fujimoto et al. 2010, Phuong et al. 2011). Concerning Palaearctic species, too few samples have been obtained through this study in order to have a clear picture of their role ($n=2$, 1, and 44 respectively for BAR, STR and MAN with only 8 positives in

MAN). However, the 17% prevalence estimated for *Cha-Pal* in MAN (n=35) indicates the need for more information about this group in particular.

This study has been implemented to provide the first longitudinal AIV information in these ecosystems. Its design was similar to most wild bird AIV survey, focusing on most probable reservoir groups, namely Anseriformes and Charadriiformes. As a result and as most wild bird AIV studies, there is little information concerning the rest of the bird community. However, we could here combined our sampling and prevalence data with counting data in the same bird communities and used available ornithological knowledge to allocate the large number of bird species into two EFGs in order to simplify the multi-host complexity. Our results do not highlight *Ans-Afr* as the main reservoir compared to other groups. In addition, all groups but one representing more than 15% of the community have a non-zero prevalence. Therefore, our data support the hypothesis that other bird groups including groups not usually regarded as important for AIV epidemiology do play a role in AIV epidemiology in these ecosystems. Our analysis points at which bird groups should be targeted for additional sampling in order to investigate further the multi-host complexity mainly, *RoC-Afr*, *Roc-Res*, *Cha-Pal* and *RoC-Pal*. Therefore, the EFG approach intend to reduce the complexity of 100+ multi-host systems in order to generate iteratively more precise hypotheses on the role of bird groups or species in the epidemiology of AIV.

In conclusion, these results are unlikely to be specific to Southern Africa. Here we observe significant AIV prevalence in *Ans-Afr* between ecosystems and provide hypotheses to explain these differences. It serves to highlight the fact that for various reasons (and most of them were valid at the time), previous studies overlooked the role of most wild bird species by focusing on a few orders or families. Comparing prevalence results from multiple sites (even if the sampling was done at similar time) is compromised if environmental and ecological variability is not accounted for. If one wants to explore AIV epidemiology in wild birds to

understand key issues such as HPAI strain emergence and local maintenance, the role of the avian community as a whole must be considered. Others EFs for AIV such as a reproductive EF taking into account fecundity and seasonality of reproduction could be used. In the Northern hemisphere, the proportion of juvenile in the population is a good indicator of AIV prevalence (Stallknecht et al. 1990) when in Southern Africa there is less synchrony in breeding. We believe that the EFG approach is a way forward to start exploring community-level epidemiology. There is a vast amount of ornithological data available which can help in designing sampling protocols. Furthermore, bird census requires expertise and time but usually does not cost much. Knowledge about the susceptibility for AIV at the bird species level is not an achievable goal in the near future. The present approach is an iterative process to select the best candidates for experimental studies to focus on. We therefore advocate for an increased integration between ecological and epidemiological data and for the necessity to develop adequate tools to study multi-host systems.

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Chapter Seven: Ecology of disease transmission in multi-host systems

(Chapter reference: Caron, A., de Garine-Wichatitsky, M., Morand, S. (Resubmitted after major revisions to *Ecology Letters*) Ecology of emerging disease transmission in multi-host systems)



Introduction

Predicting the next panzootic or pandemic requires the investigation of multi-host systems (Cleaveland et al. 2001, Taylor et al. 2001). SARS, Ebola, HPAI H5N1 have jumped the species barrier ultimately reaching the human species (Song et al. 2005, Webster et al. 2007, Leroy et al. 2009). Additionally, these diseases all involve wild and domestic hosts. While most significant parasites from domestic animals and humans have probably been described, there is still a large number of unidentified parasites of wild hosts which may translate into emerging diseases for human or domestic species (Hudson et al. 2006). The increased connectivity between ecosystems, artificially created by people and animal movements and human encroachment in pristine areas have resulted in new types of contacts between hosts and pathogens which were very unlikely under natural conditions. Parasites can use these opportunities to spill-over to new hosts at the wildlife/domestic/human interface. This question has attracted recent attention (Jones et al. 2008) but the scientific community struggles to predict which parasite will emerge and where (Dobson and Foufopoulos 2001, Woolhouse 2008). Here, we develop a conceptual and operational framework to identify transmission pathways at this complex interface. We adopt a multidisciplinary approach, integrating recent advances in community ecology, molecular epidemiology, evolutionary biology and social network analysis, shifting the research focus from the host or the pathogen to the transmission process *per se*.

Critical advances in ecology and epidemiology

Community ecology aims at understanding the rules governing species assemblage in communities (Poulin 2007a). Factors influencing parasite species composition differ between host infracommunities (individual level), component communities (population level) and

parasite fauna (species level) (Guégan et al. 2005, Poulin 2007b). We focus here on component community in host populations at the ecosystem level without making any difference between populations from different species. This level of analysis is necessary to follow transmission pathways between hosts' populations. A component community is influenced by several factors: a) hosts characteristics all influencing the diversity, quantity and exposure of the hosts' populations to parasites (body size, home range and activity); b) phylogenetic and geographic distance between hosts' populations; c) biotic and abiotic factors influencing host species richness and composition (e.g. fragmentation of the landscape, climate). The recent developments in parasite community ecology (summarized in Thomas et al. 2005, Collinge and Ray 2006, Poulin 2007b) provide an analytical approach to compare parasite communities between hosts' populations (Table 7.1). Most of the studies on parasite community ecology do not focus on transmission processes *per se* albeit these processes are at the core of the phenomenon observed. Therefore, little information has been produced on the dynamics and the temporal dimension of these parasite communities at the ecosystem level (Pedersen and Fenton 2007).

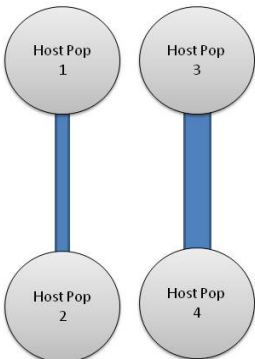
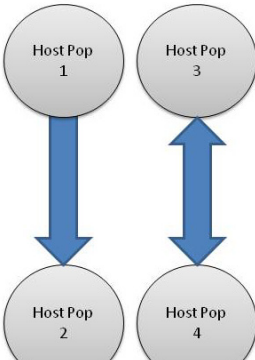
Recent developments in molecular techniques and subsequent availability of genetic information for both hosts and parasites have opened new perspectives to understand host-parasite relationships (Grenfell et al. 2004, Fricke et al. 2009, Haagmans et al. 2009). A dynamic dimension was added to molecular techniques when they were integrated with evolutionary biology (Galvani 2003). Holmes (2007a) emphasised the “research boulevards” ahead of us: co-infection interactions, intra- and inter-host viral evolutionary changes and genome wide interactions. The HPAI H5N1 case is illustrative of the power of these new technologies at hand: the diversity of strains has been used to infer geographical spread of the pathogen across the globe (Cattoli et al. 2009, McHardy and Adams 2009). Phylogenetic dynamics (phylodynamics) are increasingly integrated into quantitative epidemiology by

using concepts borrowed from evolutionary biology (Johnson and Stinchcombe 2007). As the rate of evolution is usually higher for parasites compared to their hosts, parasite genomic is relevant to trace back recent relationship between a host and its parasites (Gonzalez et al. 2007a), but also between populations of hosts (Poss et al. 2002, Biek et al. 2006, Chessa et al. 2009). This type of inference is increasingly used to explore transmission processes for specific pathogens (Heeney et al. 2006, Biek et al. 2007, Gilbert et al. 2007) (Table 7.1).

Application of network analysis to epidemiological data has attracted recent interest (Bansal et al. 2007, Heath et al. 2008). Networks represent contacts (edges) between host individuals or populations (nodes) and the parameters classically computed to characterise the network have an epidemiological significance (Luke and Harris 2007). The network properties inform on the diversity of contacts between host populations and epidemiological inference can be made (Luke and Harris 2007). Once the heterogeneity of contacts between hosts is estimated, the network provides a framework to investigate parasite spread in a defined system. In a specific context, this allows the identification of key nodes for surveillance and control (Waret-Szkuta et al. 2010).

In this paper, we hypothesise that an interdisciplinary research framework using community ecology, molecular epidemiology and network analysis can provide new insights in the understanding of disease transmission in multi-hosts systems. The application of this operational framework should contribute to the identification of the most likely pathways for future parasite emergence.

Table 7.1: Properties of epidemiological interactions between two host populations and references (illustrative, not exhaustive) for methods potentially needed for epidemiological interaction networks.

Property	Network representation	Estimation	References using relevant methodology
Intensity 	Width of edge	<p>Contact rate between host populations</p>	<ul style="list-style-type: none"> - At individual level (Courtenay et al. 2001, Cross et al. 2004, Bohm et al. 2009, Brook and McLachlan 2009, Butt et al. 2009) - At population level (Richomme et al. 2006, Dent et al. 2008, Waret-Szkuta et al. 2010) - At community level (Caron et al. 2010)
Direction 	Arrow on edge (uni- or bidirectional)	Phylogenetic analysis for one or more parasites	<ul style="list-style-type: none"> - Linking parasite population dynamics and phylogeny (Holmes and Rambaut 2004, Real et al. 2005, Hysa 2006, Bryant et al. 2007, Gilbert et al. 2007, Cottam et al. 2008b, Cattoli et al. 2009) - Inferring host populations dynamics from parasite molecular data (Poss et al. 2002, Biek et al. 2006, Koehler et al. 2008, Chessa et al. 2009)

Conceptual and operational framework

At the ecosystem level, the proposed framework focuses on one target population, as defined by Haydon et al. (2002) (e.g. human, livestock or endangered wild populations) which represents the host population at risk from disease emergence. The identification of all hosts' populations interacting with the target species potentially representing a source of parasites is a crucial step. However to date, the lack of framework for this selection process has often resulted in empirical decision-making. Parasite spill-over between two host populations is more frequent when they are phylogenetically closely related (Nunn et al. 2003). However, epidemiological investigations of recent emerging infectious diseases (EID) have demonstrated that parasite spill-over can involve distantly, related species: rodents and bats represent more than half of mammal species and have been involved in recent EIDs affecting humans (Gonzalez et al. 2007a, Klein and Calisher 2007, Leroy et al. 2009). In fact, it appears that all species interacting, even individually with the target species are relevant candidates as source of EID in a given ecosystem. In addition, disease emergence in a new species often result from complex processes, with several different species involved in the maintenance, the amplification and/or the spread of the parasite. Epidemiologists are thus confronted with an array of (sometimes loosely) interacting species, and belonging to diverse taxonomic groups, which may play a functional role in the transmission of pathogens to the target species. In Box 7.1, we present the concept of “epidemiological functional groups” (EFGs) to structure and standardise this selection process. We draw a parallel with the approaches adopted by community ecologists to assign species to functional groups and elaborate on the concept of EFGs to which hosts' species could be assigned according to their potential role in the transmission of diseases to a target species.

Box 7.1: *Epidemiological Functional Groups*

Functional ecology focuses on the functions that species play in a community (Calow 1987) (e.g. savanna's herbivores, ground-dwelling invertebrates) and functional groups of species are defined to address key process-oriented ecological questions (Simberloff and Dayan 1991). We adopt a similar approach with host communities, proposing to allocate the species coexisting in a given ecosystem into epidemiological functional groups (EFG) according to their specific life-history traits and the role they play in the transmission of a parasite, or a group of parasites, in this ecosystem.

The approach first requires a clear identification of the parasite, or group of parasites, at stake (e.g. RNA virus, *Mycobacterium* bacteria) and its mode of transmission between hosts (direct contact, vector-borne or through the environment). All (known) species potentially interacting with the target species (e.g. human, livestock, endangered wildlife) are then allocated to groups defined according to their potential role in the transmission processes: reservoir (primary) host, link (or spreader) between reservoir and target, amplifier host, and incidental (dead-end) hosts.

All species allocated to a given EFG therefore play similar roles in transmission pathways or epidemiological interactions in a particular ecosystem. To a certain extent species are thus allocated to EFG independently from taxonomic considerations and mostly based on ecological considerations as they share (at least temporarily) some resources with target and reservoir species. The example below illustrates how species can be allocated to EFG, and how, even with incomplete or inconclusive epidemiological data, this approach can help identifying key species for an identified transmission pathways.

Leroy et al. (2005) have explored the transmission pathways of Ebola virus in Central Africa. Fruit-eating vertebrates congregate on fruiting trees, a seasonal and discrete resource

in rainforest. This gathering is a potential explanation for Ebola transmission through bat saliva left on half-eaten fruits, dropped on the forest floor and subsequently eaten by great apes, monkeys or duikers. Human beings are thought to get infected when they eat or manipulate these animals. Gonzalez et al (2007b) further provided serological and molecular evidence of Ebola infection of a number of wild and domestic hosts in Central Africa. In this case, host species could be allocated to the following EFGs: fruit-eating bats reservoir, fruit-eating links (e.g. wild primates, some antelopes and livestock such as pigs), fruit-eating dead-ends (e.g. shrew, rodents or birds which are not hunted and consumed by human) and, non fruit-eating animals (e.g. wild and domestic carnivores).

- End of the Box -



The parasite emergence that one wants to predict or control is the result of the transmission of a parasite from a reservoir or intermediate host to the target species. The transmission pathway used by this parasite will depend on host mobility resulting in contacts between hosts: direct contact (e.g.: transmission through aerosol or physical contact) or indirect contact (e.g.: through a shared habitat or *via* a food resource). Not all contacts will result in parasite transmission and there is a limited number of transmission pathways between two host populations which depend on the frequency and intensity of contacts between hosts' populations. Epidemiological interactions at the ecosystem level can be presented *via* networks with edges representing the sum of transmission pathways between two nodes (or host populations). Such a network provides hypothetical pathways for future pathogen spill-over in this ecosystem. Two types of data can be used to build epidemiological interaction network: data on host ecology and data on pathogen co-occurrence in host populations.

Ecological data has already been used to estimate host contacts using telemetry, counts or direct observations (Morgan et al. 2004) (see Table 7.1). The main weakness of these techniques is that they underestimate contacts between hosts, as only a fraction of hosts' populations can be equipped or observed. In addition, the detection of host contacts does not necessarily imply the transmission of parasites (Real and Biek 2007) and the conclusive determination of infecting contacts is often difficult without an experimental and controlled design. Furthermore, few studies have focused on the contacts at the wildlife/domestic interface.

We suggest another approach based on the comparison of parasite component communities shared by sympatric hosts' populations, which can be considered as an indicator of past host contacts successful in transmission events. In other words, the shared parasites indicate the extent of the epidemiological interactions which have occurred between two

hosts' populations. At the ecosystem level, the pair wise shared community of parasites between populations can be used to build networks of epidemiological interactions between hosts' populations. The assumption we make is that successful transmission pathways used by some parasites in the past could also be used by other pathogens, especially if they share the same mode of transmission. In addition, the more parasites with different modes of transmission shared by two host populations, the higher the probability of the epidemiological network to have identified a future transmission pathway.

Interaction networks have been used for public and animal health studies (Bansal et al. 2007, Dent et al. 2008, Heath et al. 2008, Waret-Szkuta et al. 2010). Classically the nodes represent host populations and the edges represent the epidemiological interactions (see for a definition Chapter Three - Caron et al. 2010) between populations. Parasite component communities define the properties of each node and the shared parasites between host populations determine the two main properties of edges: their intensity and their direction (uni- or bidirectional) (see Table 7.1) (and see an example in Box 7.2).

The intensity of the edges can be estimated using direct estimation of host contacts or similarities of component communities (Poulin 2003, Vinarski et al. 2007, Krasnov et al. 2009, Poulin 2010). Community ecology studies have investigated the decay of similarity between parasite communities with phylogenetic or geographic distance (Poulin 2007b). Phylogenetic distance between host populations and sampling effort need to be controlled for, and appropriate methods are currently developed (see references in Table 7.1). By design, the geographic distance between host populations is accounted for as they belong to the same ecosystem but spatially explicit epidemiological networks have also been designed (Poulin 2007b). Qualitative and quantitative methods have been developed to measure component community similarity: the Jaccard Index (Jaccard 1912) is a simple presence/absence index; the Sorensen (Vinarski et al. 2007) and Morisita-Horn index (Horn 1966) are quantitative

indices using proportion of different parasites or abundance (i.e., prevalence data in epidemiology). The diagnostic methods used for parasite detection are also of importance as they do not all detect the same indicator of parasite presence (e.g. antibodies, antigens). If most available studies have used direct observation of macroparasites during post-mortem inspection which is assumed to be both sensitive and specific, for most microparasites however, direct observation is not an option and specific detection techniques need to be applied.. The index values calculated by comparing pairs of parasite component communities (nodes) characterise each edge of the network (Table 7.1).

The direction of the interaction between two hosts' populations indicating which host population is at the origin of the parasite transmission, cannot be measured with the community ecology approach presented above. A priori, epidemiological interactions are bidirectional, as direct contact between two hosts can potentially result in parasite transmission both ways. However, transmission related to host contacts can be asymmetric: when a reservoir host transmits a parasite to a naive population or when the differential use of a habitat translates into indirect parasite transmission. The concepts and tools of population genetics and parasite genomics may help at tracking back the direction of transmission. Phylogenetic trees have appeared in epidemiological literature and successions of outbreaks can be followed based on variations in parasite genomes (Table 7.1). Most of these tools have been applied to parasite species of economic or public health importance such as HIV (Heeney et al. 2006), foot-and-mouth disease (Cottam et al. 2008a) or tuberculosis (Michel et al. 2008). Information on these parasites can be added in edges of the interaction network and inform the network on transmission pathways of neglected parasites sharing an EI.

Box 7.2: *Epidemiological Interaction Network for 14 rodent species and the human species.*

In this example, the human species is our target species and we explore the Epidemiological Interactions (EIs) between the human species and several rodent species present in particular ecosystems of Southeast Asia represented by different habitats (dry and irrigated agricultural areas, forests, and villages).

From the literature (Chaisiri et al. 2010, Herbreteau et al. unpublished), we obtained presence-absence data on 14 rodent species. Information about 34 macroparasite species and 8 microparasite species were collected for these 14 rodent species and susceptibility to these parasites for the human species were taken from the available literature (Table 7.2). A 42 parasites*15 hosts matrix was built (and filled with “1” or “0” for occurrence of infection and absence respectively in each host species. This matrix was used to calculate the Jaccard Index (=number of parasite species present in both host populations/sum of parasite species present in each host populations) displayed in Table 7.3. The Jaccard index value varies therefore between “0” for no parasite species shared and “1” for all parasite species shared.

We used the Jaccard index as a proxy of EIs between each host population and built the corresponding EI network (Figure 7.1).

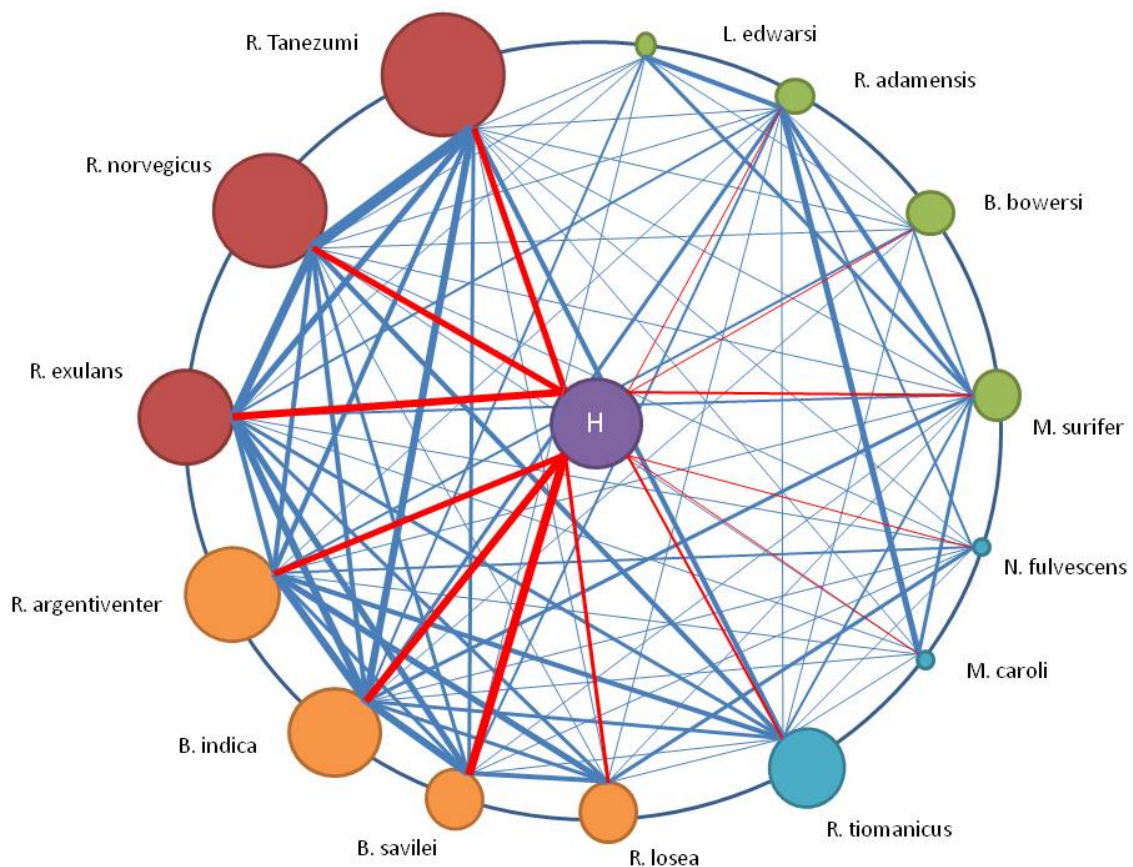
Table 7.2: Host and parasite species used

Target sp.	<i>Homo sapiens</i>
Rodent sp.	<i>Bandicota indicata</i> (Bi), <i>Bandicota savilei</i> (Bs), <i>Verylmys bowersi</i> (Bb), <i>Leopoldamys edwardsi</i> (Le), <i>Maxomys surifer</i> (Ms), <i>Mus caroli</i> (Mc), <i>Niviventer fulvescens</i> (Nf), <i>Rattus andamanensis</i> (Ran), <i>Rattus argentiventer</i> (Rar), <i>Rattus exulans</i> (Re), <i>Rattus losea</i> (RI), <i>Rattus norvegicus</i> (Rn), <i>Rattus tanezumi</i> (Rta), <i>Rattus tiomanicus</i> (Rti)
Macroparasite sp.	<i>Hymenolepis nana</i> , <i>Rodentolepis</i> sp., <i>Taenia</i> sp., <i>Taenia taeniaeformis</i> , <i>Ascaris</i> sp., <i>Gnathostoma malaysiae</i> , <i>Ganguleterakis spumosa</i> , <i>Citellina levini</i> , <i>Syphacia muris</i> , <i>Physaloptera</i> sp., <i>Rictularia</i> sp., <i>Rictularia tani</i> , <i>Gongylonema neoplasticum</i> , <i>Mastophorus muris</i> , <i>Protospiura-Mastophorus</i> sp., <i>Cyclodontostomum purvisi</i> , <i>Strongyloides ratti</i> , <i>Strongyloides</i> sp., <i>Nippostrongylus brasillensis</i> , <i>Nippostrongylus</i> sp., <i>Orientostrongylus tenorai</i> , <i>Echinostoma ilocanum</i> , <i>Echinostoma malayanum</i> , <i>Notocotylus</i> sp., <i>Quinqueserialis quinqueserialis</i> , <i>Gastrodiscoides hominis</i> , <i>Centrocestus</i> sp.
Microparasite sp.	Leptospirosis, scrub typhus, Bartonella, hanta virus, herpes virus, LCM virus, Trypanosoma, rabies virus.

Table 7.3: Matrix of Jaccard index values between 15 host species for a shared community of 42 pathogens. “ID” represents the host species name abbreviation (Hs = *Homo sapiens*). ‘Nb Patho’ indicates the number of pathogens detected in each host species.

	Nb Para	Bi	Bs	Bb	Le	Ms	Mc	Nf	Ran	Rar	Re	RI	Rn	Rta	Rti	Hs
Bi	16															
Bs	9	0,47														
Bb	7	0,15	0,00													
Le	3	0,12	0,09	0,11												
Ms	8	0,20	0,06	0,15	0,22											
Mc	2	0,06	0,00	0,00	0,00	0,25										
Nf	2	0,06	0,10	0,00	0,00	0,00	0,00									
Ran	5	0,24	0,17	0,09	0,33	0,30	0,40	0,17								
Rar	17	0,43	0,24	0,14	0,00	0,09	0,06	0,12	0,10							
Re	18	0,48	0,35	0,00	0,11	0,13	0,05	0,05	0,15	0,30						
RI	9	0,19	0,29	0,00	0,00	0,06	0,00	0,22	0,08	0,37	0,23					
Rn	23	0,34	0,23	0,03	0,04	0,07	0,04	0,09	0,08	0,33	0,52	0,19				
Rta	32	0,45	0,24	0,11	0,06	0,14	0,03	0,06	0,09	0,32	0,47	0,17	0,72			
Rti	13	0,26	0,05	0,05	0,00	0,17	0,07	0,07	0,06	0,36	0,24	0,10	0,33	0,36		
Hs	15	0,48	0,50	0,05	0,06	0,21	0,06	0,06	0,18	0,28	0,43	0,20	0,41	0,42	0,17	

Figure 7.1: *Epidemiological Interaction Network for 14 rodent species and the human species in the Southeast Asian ecosystems based on presence-absence data for 34 macroparasite species and 8 microparasite species. Each node represents a host species, the size of the node is proportional to the number of parasite species harbored by the host and the color of the circle represents the habitat in which the host species is mostly found (except for human): red=in human settlements; orange=in rice fields; blue=in modified forest and dry agricultural areas; green=in primary forest. Each edge between two nodes represents the shared parasite community and its width is proportional to the Jaccard index. We placed the human species in the centre of the figure and its edges in red for visual comfort.*



The analysis of this network leads to the following observations:

- Interpreting the size of the nodes, the three rodent species with the highest parasite diversity are occurring in human settlements. The three rodent species with the lowest parasite diversity occur in primary or secondary forest and dry agricultural land.
- The size of the human species node indicates that we share 15 parasite species with rodent species studied here.
- Interpreting the width of the edges at the network level, there is a higher density of large-width edges on the left of the network, indicating that rodent species in human settlements and rice-fields share a higher proportion of their parasite diversity than rodent species in the remaining habitats.
- Interpreting the width of the edges concerning the human species, Bi and Bs have the highest Jaccard index values (0.48 and 0.5 respectively), followed by Re, Rta and Rn (0.43, 0.42, 0.41 respectively).
- The nodes of Bi, Ran, Rn and Rta have the maximum number of edges ($n=14$) possible in this network. They all occur in human settlements, rice fields except for Ran occurring in primary forest. The nodes of Bb, Le and Mc have the lowest number of edges in the network ($n=9$) and they all belong to primary and secondary forest or dry agricultural areas.
- The node of the human species has 13 edges close to the maximum of 14.

This preliminary analysis of the EI network provides more information than a separate analysis of each parasite species and their hosts. The method of calculus of the index needs to be kept in mind: the observation that Bi and Bs have the highest Jaccard index values with the human species is irrelevant as the human species shares more parasite species with Rn and Rta than with Bi and Bs (11, 14, 10, 8 respectively). The difference is due to the high parasite

species richness of R_n and R_{ta} . Other indices can be used to address this kind of issue but no index is perfect. However, this first network can orientate surveillance protocols towards the most interesting host species to be included in order to answer the question at stake: if the question is the probability of emerging infectious diseases in humans from rodent hosts in this ecosystem, the surveillance protocol will target species living in the human settlements (and a ranking can be done on this species) and in the rice fields with maybe R_{ti} being an interesting sentinel species to look at as a bridge between pristine and modified environment. To our knowledge, this species is never mentioned as a potential source of infectious disease or as a potential sentinel for disease surveillance in the literature.

- End of the Box -



The temporal variability of the interaction between two host populations can also be obtained by molecular analysis on specific parasites in the different populations or longitudinal studies designed for detecting parasite seasonal profiles. An interaction network can change drastically between seasons as host contacts will vary with host movements depending on host ecology and resource availability (Brook and McLachlan 2009, Butt et al. 2009). This temporal dimension can be represented by different networks for different seasons.

Scope and limitations of the approach

We believe that the definitions and the framework presented in this paper can provide a solid basis to explore the ecology of disease transmission in multi-host systems as it disentangles the complex processes involved in transmission and provide further testable hypotheses. However, there are several limitations which should be kept in mind when interpreting epidemiological interaction networks.

First, host susceptibility to specific parasites is important to consider as it can blur the directionality of epidemiological interactions between two nodes/hosts' populations. A parasite not shared by two populations could be the result of the host lacking susceptibility for this parasite. Co-evolved host-pathogen interactions result in a more stable network (in time) than recently created interactions. Most wildlife/domestic/human interfaces are the products of recent changes in human activities or behaviours (Daszak et al. 2000, Osofsky et al. 2005) and the newly established epidemiological interactions are possibly in an unstable state in time. Second, inter-parasite ecological interactions within hosts (e.g. direct competition or synergies or indirect through the host immune system) can influence epidemiological interactions networks although this is a poorly explored field of research (but

see Poulin 2005, Jolles et al. 2008, Lagrue and Poulin 2008, Telfer et al. 2010). These ecological interactions can provide another explanation for the lack of detection of a parasite in a susceptible host population: its elimination by direct or indirect competition by another parasite. Third, the performance of the diagnostic tests used need to be assessed: parasite isolation and antibody detection techniques do not give the same information about the past and present history of host-pathogen interactions. Whenever possible, this type of data should be harmonised across parasites.

Fourth, the variability in transmission modes across parasites in relation with EFGs will have a high impact on the network. Therefore the choice of the parasite species under study and the definition of EFG relevant to the transmission mode of this parasite will be crucial. This choice can be guided by knowledge of the parasite suspected to emerge and the life history traits of potential hosts in the ecosystem. RNA viruses are good candidates due to their implication in recent emergence (Cleaveland et al. 2007, Holmes and Grenfell 2009). If no *a priori* is made about the future emerging parasite, we suggest after building the global epidemiological interaction network, to provide subsets of this epidemiological interaction network based on transmission modes. The comparison of these networks can help identifying particular properties related to specific transmission modes. For a more holistic approach, the parasite choice should be oriented towards species representative of the different transmission modes in the ecosystem.

EIDs at the wildlife/domestic/human Interface

The common context of the wild/domestic interfaces from an ecological perspective is:

- a) a multi-host system, increasing in complexity as wildlife diversity increases;
- b) a multi-parasite system, increasing in complexity as wildlife diversity increases;
- c) the type of

interface (e.g. fence, area of contact), in expansion worldwide and creating a mosaic of contrasted natural and human-modified habitats. EIDs have recently captured the attention of media and scientific community (Cleaveland et al. 2007, Alexander and McNutt 2010). The recent steep increase in the power of technical (molecular) tools and the multiplication of emergence events in a globalised and changing world have increased the perceptions of EIDs as a threat for animal and public health. Several reviews have identified potential “hotspots” for parasite emergence (Jones et al. 2008, Woolhouse 2008) and underlined the linkages between human, domestic and wild parasites (Cleaveland et al. 2001, Taylor et al. 2001, Jones et al. 2008). Multi-steps processes have been presented to offer a mechanistic framework for emergence events (Woolhouse et al. 2005, Childs et al. 2007, Wolfe et al. 2007, Lloyd-Smith et al. 2009) *sensu stricto*. The emerging pathogen is detected in a target species with a variable time-lag between the inter-species transmission and the detection. This time-lag associated with ecological traits of the parasite and host will determine the severity of the outbreak. For instance, the time-lag for AIDS has taken probably several decades and maybe centuries from the first human case to the recognition of the disease at the beginning of the 80’s (Heeney et al. 2006, Holmes 2007b); for Ebola, the time-lag has often been short with massive localised human deaths (Leroy et al. 2009); finally for SARS both detection and spread have been quick (Rota et al. 2003). A smaller time-lag between interspecies spill-over and detection can save lives and limit the socio-economical impact of EID outbreak (Childs and Gordon 2009). From a scientific, ethical and economical point of view, research, surveillance, prevention and control should focus on EID hotspots in order to anticipate and prevent epizootics or epidemics potentially leading to panzootics and pandemics.

We believe that the epidemiological interaction networks can provide the basis for reducing the time lag between actual spill-over of pathogens and detection in EID hotspots. We propose a framework for the selection of hosts’ populations allocated to EFGs which

should be monitored in priority in a given hotspot. By identifying and quantifying epidemiological interactions between hosts' populations, a risk can be attributed to each transmission pathways. Epidemiological interaction networks generate testable predictions of future parasite emergence, with direct implication for surveillance and control in a resource-limited environment. From a practical point of view, EID hotspots are usually located in remote areas of developing countries, economically poorly developed. Using already available sanitary information (e.g. from governmental veterinary services, NGOs) can provide the data to start building a network helpful in identifying gaps of knowledge or key hosts' populations or parasites.

The EI network framework that we present here could achieve two objectives: increasing theoretical knowledge on the ecology of disease transmission and on multi-host multi-pathogen interactions and providing a tool for EID early detection. A crucial question in the ecology of disease transmission will be to determine if EFGs share common properties (see Box 7.1). Are there common transmission processes for parasites with different modes of transmission? And do hosts species play similar functional epidemiological roles for different parasites? If transmission processes in a given ecosystem share generic properties, these findings will have important consequences on animal and human health surveillance and control as resources could be more efficiently targeted for priority host populations and transmission chains.

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Chapter 8 – General Conclusions

Alexandre Caron



In this thesis, I defined the concept of epidemiological interaction (EI) between two host populations and presented two approaches to estimate these EIs. The first approach (Chapter Two - Caron et al. 2009) assumed that the movements of hosts and the contacts induced by this mobility would estimate EIs. The second approach (Chapter Seven & Appendix Six) assumed that a limited quantity of potential transmission pathways exist between two host populations (e.g. direct contact, vector transmission) and that past occurrence of transmission could estimate future occurrence (disease emergence).

In the framework of this thesis, I mostly developed the first approach. Using the model of wild and domestic avian communities in a Zimbabwean wetland, I gathered regular bird count and pathogen prevalence data during two years in order to provide information about the ecology of AIV in a multi-host system and tested my assumptions on the first approach (Chapter Three, Four, Five & Six - Caron et al. 2010, Caron et al. 2011). The main outcomes of this approach were the new sanitary information provided. I suggested for the first time a persistence of AIV in an African ecosystem based on multi-species data. I provided the first occurrence of AIV in some waterfowl species and a solid three-year dataset of ornithological data. Secondly, I explored how a host community approach can help define risk season and risk species for both the epidemiological cycle (reservoir, spreader, maintenance host) and at the wildlife/domestic interface (bridge species). Chapter Three, Four & Five (Caron et al. 2010, Caron et al. 2011) explained the difficulty of estimating prevalence in multi-host systems and how this complexity could be used to provide integrated ecological and epidemiological tools improving the prevalence estimation. And finally Chapter Six presented how ecological and epidemiological data could be integrated in a risk analysis approach to estimate the risk of pathogen spread through wild birds at the wildlife/domestic interface in order to provide stakeholders with data-based decision making tools for limited-resource allocation. Articles included as appendices showed how this work was integrated in a broader

framework of research on the ecology of avian influenza viruses in African waterbirds at the regional and continental level (Appendix One, Two, Four & Five - Gaidet et al. 2007a, Gaidet et al. 2007b). In parallel, the same approach was used on wild/domestic ungulate community in the South-East Lowveld of Zimbabwe, trying to explore transmission pathways for bovine tuberculosis and other important diseases (Appendix Three - de Garine-Wichatitsky et al. 2010). This additional model provided the opportunity to explore host-pathogen interaction in a different system with a different host approach; we detected the spread of bovine tuberculosis from South Africa to Zimbabwe and established a telemetry study at the wildlife/livestock interface exploring the host approach at the population level with a social network analysis perspective (manuscript in preparation). The juxtaposition of these models highlighted the process-centred approach that I focused on instead of a host- or pathogen-centred approach: I (in collaboration with my colleagues on the second model) explored and tested the same hypotheses in the two models.

The second approach to estimate EIs was mostly developed in the Chapter Seven and Appendix Six. Here we presented a conceptual and operational framework and used the shared community of parasites between fourteen rodent species and the human species to build an interaction network. We compared it with a similar network built using trapping data to estimate host co-occurrence. We discussed the advantage and weakness of this approach and explain in which circumstances it could be used. To my mind, this method could be used as an on-going process: in a given ecosystem, ecological and epidemiological available data (e.g. through national surveillance system or NGO projects) could be integrated in a preliminary interaction network. This network could help identify the gaps in information and be re-enforced once this information is produced. This chapter had therefore two sides: a purely fundamental background rooted in the ecology of disease transmission; and an applied aspect able to help surveillance systems to identify and source the right information and

provide health managers with control options for emerging diseases. In the near future, the two models developed in this thesis (the avian and ungulate models) will provide the data needed to validate this approach empirically. Wild birds sampled have been or will be tested for Newcastle disease, West Nile Virus and Avian Malaria. In addition, domestic and wild ungulates have been tested for bovine tuberculosis, Foot-and-Mouth Disease, Rift Valley Fever, Brucellosis, the most common tick-borne diseases (theileriosis, babesiosis, anaplasmosis), Lumpy Skin Diseases and faecal macroparasites.

From a critical point of view, if this thesis has developed new analytical tools at the interface of different scientific fields (epidemiology and ecology), it was confronted to a level of complexity (multi-host, multi-pathogen systems) not fully integrated in the products. This thesis should be seen as opening new avenues for future research rather than delivering a final output. Furthermore, the level of analysis of this work is embedded in lower levels of host-pathogen interaction, at the individual level and at the molecular level (e.g. immunology, molecular epidemiology) which should be controlled for or at least acknowledge when analysing eco-epidemiological data with the approaches presented here.

The complexity of multi-host systems prevents a clear-cut analysis of host and pathogen interaction at the host community level. The range of possible interactions between hosts and between pathogens becomes quickly impossible to track. However leaving aside the host community level is detrimental to epidemiological studies. Haydon et al. (2002) present different configurations of a multi-host reservoir. Surveillance or control management options missing an important host in a multi-host reservoir will fail. Similarly, Telfer et al. (2010) recently demonstrated how interactions between pathogens in a host population can bias the results of single-pathogen studies. Both these studies are important because of the two major shifts in wildlife/domestic epidemiology they induce: 1) most data produced to date have to be interpreted with caution as underlying interactions between parasites or influence by

unknown hosts can interfere with the direct host-parasite interaction observed; 2) analytical tools to estimate real host-pathogen interactions based on limited ecological and epidemiological information are not yet available or not yet integrated between the various fields of ecology and veterinary sciences. This is important to realise as our focus concerning emerging infectious diseases is now turning towards epidemiological systems embedded in this complexity at the wildlife/domestic/human interface (Cleaveland et al. 2001, Taylor et al. 2001). Most emergence events will occur in multi-host systems hot spots (Jones et al. 2008). This could be seen as a rather pessimistic perspective for animal and human health. I believe it is not. Efforts will be necessary to produce the interdisciplinary mixing, needed between researchers but also for individual profiles through proper academic careers. The research community already has tools available to analyse this complexity which needs to be integrated across different thematic fields. I have tried to show in my thesis how the integration of tools borrowed from community ecology and epidemiology bring more information than a separated analysis by each field. As presented in the last chapter of this thesis, evolutionary biology feeding molecular epidemiology can also help in identifying the direction of transmission pathways. However, the technological power necessary to have an exhaustive study at the ecosystem level of hosts and parasites species is not yet to hand.

Today, epidemiologists and health ecologists face the dilemma faced by ecologists when they apprehended the complexity of the study of organisms' interactions at the community level: the impossibility of taking into account the full spectrum of host-pathogen interactions at the community level with the tools available. However, patterns and general rules can still be extracted from the complexity and I hope this thesis can provide concepts and frameworks to reach this objective.

However, in a final futuristic comment, I would state that I think that the ecology of disease transmission or the study of epidemiology at the host-pathogen community level will

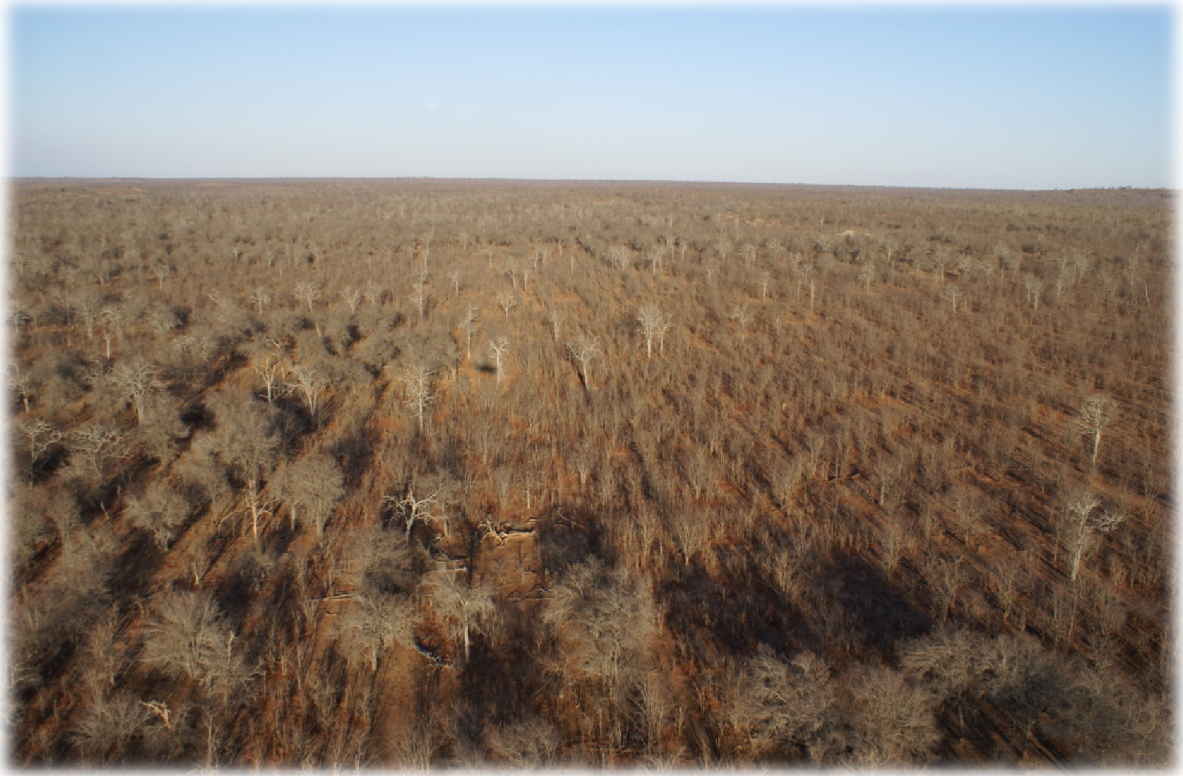
soon have a net advantage on community ecology investigating ecological interactions between non-parasite organisms. When a host-pathogen interaction occurs, it leaves a “trace” in the host and/or in the pathogen. This trace can be: 1) the presence of the pathogen or of products of the immune system responding to the presence (or past presence) of the pathogen; 2) it can also be a change in the genome of the host or the pathogen (induced mutation, evolutionary response to the interaction). These traces of host-pathogen interaction can be used at the community level as a history of epidemiological interactions. As an example, the development of non-invasive techniques to detect the presence of pathogens in faeces is providing professionals with an amount of available data with low cost of sampling (no need of physical capture for this type of wildlife sampling). The recent development of a broad spectrum genetic material detection machine – broad range acid nucleic sequencer - (Gupta et al. 2009, Holmes and Grenfell 2009) makes clear the point that it is already possible to extract from any substrate (e.g. tissue, faeces) the entire community of acid nucleic sequences. This broad spectrum detection refers to known and unknown acid nucleic sequences. So far, we lack the power needed to analyse and segregate such a huge amount of data. In a few years, we will have at hand those analytical tools.

In conclusion, I believe this thesis explored ways to further development in the ecology of disease transmission. By addressing questions at the transmission process-level, it explores what could be the general properties and/or rules governing disease transmission, independently of the pathogen at stake. There may be none, but if they exist, they could change some current practices in disease management.

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Appendix One: Avian influenza A viruses in waterbirds in Africa

(Appendix reference: Gaidet, N., Dodman, T., Caron, A., Balança, G., Desvaux, S., Goutard, F., Cattoli, G., Hagemeijer, W., Monicat, F. 2007. Influenza A viruses in waterbirds in Africa. *Emerging Infectious Diseases*, 13 (6): 626-629)



Introduction

Wild waterbirds are considered to be the major natural reservoir for influenza A virus (IAV) (Webster et al. 1992). Large numbers of Eurasian breeding waterbirds over winter in the sub-Saharan region of the African continent (Del Hoyo et al. 1996), where the survival of IAV is considered to be restricted by tropical environmental constraints (Stallknecht et al. 1990). There is however a knowledge gap in the ecology of IAV in tropical regions (Webster et al. 1992, Olsen et al. 2006): it is not known if IAVs circulate in waterbird communities in Africa and if tropical ecosystems can have a role in the perpetuation of IAV among waterfowl. In this study, we report about results from a large-scale surveillance in waterbirds conducted in 12 countries over the African continent (Figure A1.1).

The Study

This surveillance programme was implemented in early 2006 within the framework of FAO's Technical Cooperation Programmes of Emergency Assistance for Early Detection and Prevention of Avian Influenza. We conducted field sampling operations in partnership with national experts from wildlife and veterinary services, and in collaboration with international conservation and research organisations (AFRING, OMPO, ONCFS, SOVON, WWT), local ornithological NGOs, as well as national hunting associations and safari operators. Study species were selected among bird families recognised as major IAV reservoirs (notably Anseriformes and Charadriiformes), in both Eurasian and Afro-tropical bird communities. Study sites were selected in key sites for congregatory waterbirds, including those where Palearctic and Afro-tropical birds mix, in accordance with national surveillance programmes and field logistic constraints.

Figure A1.1: Locations of sampling sites (or cluster of sites) in surveyed countries (dark grey) initially participating in the FAO's Technical Cooperation Programmes (light and dark grey).

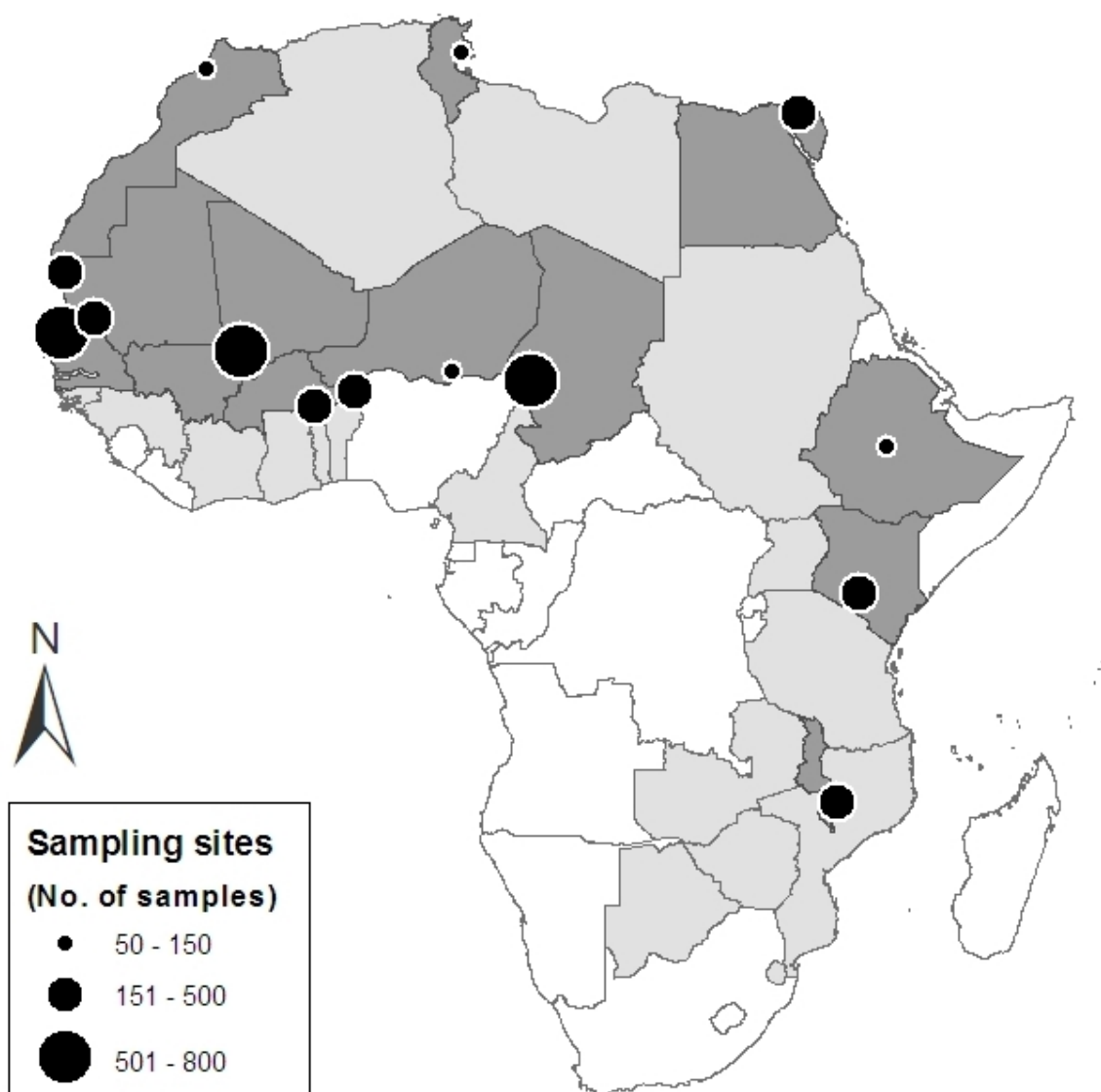


Table A1.1: Prevalence of influenza A virus in wild birds detected by RT-PCR.

Bird group (No. species tested)	No. samples tested	No. PCR- Positive (%)	RT-PCR positive bird species	Originating countries of positive samples
African ducks (9)	1455	41 (2.8)	<i>Dendrocygna viduata</i> <i>Sarkidiornis melanotos</i>	Chad, Mali, Ethiopia, Mauritania, Niger, Senegal
Eurasian ducks (6)	1371	89 (6.5)	<i>Anas acuta</i> <i>Anas querquedula</i>	Chad, Mali, Mauritania, Morocco, Niger, Senegal
Gulls (3)	366	14 (3.8)	<i>Larus fuscus</i> <i>Larus genei</i>	Mauritania, Senegal
Terns (7)	159	2 (1.3)	<i>Sterna sp.**</i>	Mauritania
Eurasian waders * (13)	379	6 (1.6)	<i>Calidris ferruginea</i> <i>Philomachus pugnax</i> <i>Tringa erythropus</i>	Mali, Tunisia
Rails (7)	416	3 (0.7)	<i>Gallinula chloropus</i> <i>Porphyrio porphyrio</i>	Mali
Cormorants (2)	148	0 (0)		
Others	259	0 (0)		
Total	4553	155 ***		

* Scolopacidae

** Unidentified fresh dropping samples from a multi-species flock of *Sterna caspia*, *S. maxima* and *S. sandvicensis*.

*** excluding 4 PCR positive samples from non specified wild birds.

From mid January to mid March 2006 (and May in Tunisia), we collected cloacal swabs from captured birds and from freshly killed birds provided by hunters. Fresh dropping samples were also collected at roosting areas for gulls, terns and some ducks. In Ethiopia, where there were hunting restrictions, and in countries where emergency surveillance operations were implemented following notification of H5N1 outbreaks in Nigeria (Burkina Faso, Niger), birds were shot through special permits for sample collection (n= 732).

The transport medium consisted of an isotonic phosphate buffered saline (PBS), pH 7.0-7.4, containing antibiotics (penicillin 10,000 units/ml, streptomycin 10 mg/ml, amphotericin B 25 µg/ml and gentamycin 250 µg/ml) supplemented with 10% glycerol. Samples were stored in liquid nitrogen containers in the field, or in a classic freezer before storage in a deep freezer (-80°C), and were shipped to laboratories in dry liquid containers or cryopacks.

Samples were analysed at the Istituto Zooprofilattico Sperimentale delle Venezie (Italy), except for samples from Egypt analysed at the US Naval Medical Research Unit-3 (Egypt), from Kenya and Malawi at the Agricultural Research Council Onderstepoort Veterinary Institute (RSA) and from Tunisia at the Southeast Poultry Research Laboratory USDA/ARS (USA). The samples were all screened by real-time RT-PCR specific for type A influenza viruses (Spackman et al. 2002), and positive samples were tested by RT-PCR specific for H5 subtype. All type A positive samples were subsequently processed for virus isolation using standard methods. Briefly, 100 µl of the original sample were inoculated into the allantoic cavity of 9-10 day-old embryonated specific pathogen free eggs for virus isolation attempts according to EU Directive 92/40. Haemagglutinating isolates were characterized by haemagglutination-inhibition test and neuraminidase inhibition test using specific hyperimmune chicken antisera to the reference strains of influenza virus (Alexander

et al. 1979). Molecular pathogenicity of H5 subtype positive samples was determined by sequencing the haemagglutinin gene segment. Sequences were performed using the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems) in a 3100-Avant Genetic Analyzer (Applied Biosystems).

A total of 4553 birds were tested (Table A1.1), consisting of a majority of Afro-tropical and Eurasian ducks (32% and 30% of samples respectively). Other samples originated mostly from gulls and terns (11%), rails (9%), waders (8%) and cormorants (3%). The overall detection of IAV was 3.5 % (n=159 RT-PCR positive samples, including both cloacal swabs and fresh droppings). Low pathogenic IAVs were detected in 12 bird species of ducks, waders, gulls, terns and rails, including both Eurasian and Afro-tropical bird species (Table A1.1). Positive samples were obtained from birds collected in 8 distinct countries (Chad, Ethiopia, Mali, Mauritania, Morocco, Niger, Senegal and Tunisia). In the two most frequently sampled species, a Eurasian duck (Garganey *Anas querquedula*, n=1329) and an Afro-tropical duck (White-faced Whistling Duck *Dendrocygna viduata*, n=1157), IAVs were detected from most surveyed countries, but with a highly variable prevalence (Table A1.2). No H5N1 virus was detected, nor any highly pathogenic IAV. A total of 11 samples were positive for H5 subtype, mostly from Garganey (H5 prevalence of 0.7%). Finally, five low pathogenic IAVs could be isolated: three distinct isolates originated from Garganey sampled in the Inner Niger Delta in Mali (H5N3, H11N9, H12N5), and two isolates originated from White-faced Whistling Duck sampled in Ethiopia (H8N4) and in Senegal (H1N1).

Table A1.2: RT-PCR based detection of influenza A virus in two wild ducks sampled in various surveyed countries.

Species	Country	No. samples tested	No. PCR-Positive (%)
<i>Anas querquedula</i>	Chad	381	11 (2.9)
	Kenya	104	0 (0)
	Mali	411	22 (5.4)
	Mauritania	225	33 (14.7)
	Niger	87	4 (4.6)
	Senegal	121	17 (14.0)
<i>Dendrocygna viduata</i>	Burkina Faso	167	0 (0)
	Chad	232	1 (0.4)
	Ethiopia	76	10 (13.2)
	Malawi	59	0 (0)
	Mali	36	1 (2.8)
	Mauritania	183	7 (3.8)
	Niger	232	8 (3.4)
	Senegal	172	11 (6.4)

Conclusions

The African continent, and in particular its sub-Saharan region, constitutes a seasonal shelter for a large number of Eurasian waterbirds, including an estimated 5.4 million ducks that gather in Western and Eastern Africa during the northern winter (Dodman In Press). In their over-wintering sites, these birds congregate and mix with a wide variety of Afro-tropical waterbirds.

Results from this surveillance programme established that IAVs are present in wild birds in Africa during the northern winter. Low pathogenic IAVs were detected and isolated in wild birds in several major wetlands of Northern, Western and Eastern Africa, indicating that environmental conditions in Afro-tropical ecosystems are favourable to the persistence and transmission of IAV.

We detected and isolated IAV in both Eurasian and Afro-tropical species. This finding reveals that IAVs circulate in the migratory waterbirds originating from Eurasia, but also in the African species that remain in the continent all year long. Moreover, the detection of viruses in some Eurasian wader species, during both wintering (in January in Mali) and migration (in May in Tunisia), contrasts with the apparent absence of IAVs reported in previous studies of waders (Fouchier et al. 2003) in Europe. Waders being widely the most abundant African-Eurasian migratory waterbird group (Stroud et al. 2004), this result suggests that these shorebirds might play a significant role in the perpetuation and transmission of IAV in waterbird communities across continents.

The presence of IAV detected in Eurasian ducks in several of their major over-wintering sites in West Africa (e.g. the Inner Niger Delta, the Senegal River Delta and Lake

Chad) supports the former hypothesis that IAVs can persist in wild duck populations all year round through a continuous circulation in a proportion of birds (Webster et al. 1992). The different isolates obtained from Garganey in the Inner Niger Delta in Mali also indicate that various subtypes are circulating at the same time in a population, in agreement with patterns observed in Europe and North America (Fouchier et al. 2003, Krauss et al. 2004).

Various IAV subtypes were isolated from apparently healthy Garganey and White-faced Whistling Ducks indicating that both Eurasian and Afro-tropical ducks can serve as reservoirs of IAV. These results suggest that some Eurasian ducks are likely to be carriers of IAV on their northwards spring migration, but also raise the possibility for a potential persistence of IAV in the tropical region and dissemination over Africa through intra-African migratory ducks. The presence of IAV in African wintering and stop-over sites where birds from various geographical origins congregate and mix provide the opportunity for IAV to be transmitted between different bird populations and to be spread over extensive areas in both Eurasia and Africa



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**Appendix Two: Influenza Surveillance in Wild Birds in Eastern Europe,
the Middle East, and Africa: Preliminary Results from an Ongoing
FAO-led Survey**

(Appendix reference: Gaidet, N., Dodman, T., Caron, A., Balança, G., Desvaux, S., Goutard, F., Cattoli, G., Martin, V., Tripoli, A., Lamaruque, F., Hagemeijer, W., Monicat, F. 2007.

Influenza Surveillance in Wild Birds in Eastern Europe, the Middle East, and Africa: Preliminary Results from an Ongoing FAO-led Survey. *Journal of Wildlife Diseases*, 43: S22-S28)



Introduction

Migratory waterfowl generally are considered the natural reservoir of avian influenza (AI) virus (Olsen et al. 2006). Large numbers of waterbirds that breed in the Palearctic overwinter on the African continent. In the context of the spread of the highly pathogenic avian influenza (HPAI) Asian lineage H5N1 virus through Eurasia during summer 2005, concerns arose that this virus could be spread southward toward Africa in wild birds during fall migration. In November 2005, the Food and Agriculture Organization (FAO) set up five regional Technical Cooperation Programmes (TCP) of Emergency Assistance for Early Detection and Prevention of AI, in five regions of Eastern Europe, the Middle East, and Africa. These programmes were developed to provide on a country basis support for strengthening emergency preparedness against the potential introduction and progressive spread of HPAI H5N1 virus within these regions, specifically in relation to migration of and trade in wild birds, and the interface between wild birds and domestic poultry.

The FAO has been collaborating with national veterinary services, national wildlife institutions and international collaborating centres (**Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD)**; Istituto Zooprofilattico Sperimentale delle Venezie; Royal Veterinary College, University of London; and Wetlands International) to strengthen field surveillance and laboratory diagnostic capacities through training and capacity building. A risk analysis procedure was implemented for the development of contingency action plans to strengthen early warning of and early reaction to HPAI introduction. These TCPs also aimed to promote the development of capacity for sharing HPAI disease intelligence through the establishment of information and technology network linkages within and between the regions, in relation to the development of a global system for HPAI surveillance.

Within the framework of these TCPs, a surveillance study was launched in early 2006 to evaluate if HPAI H5N1 virus could be perpetuated in wild bird populations in countries where HPAI H5N1 outbreaks occurred or may occur considering the movement of wild birds. The objective also was to provide technical support to national surveillance programmes through capacity building, and to standardise field procedures.

Methods

Implementation of the field surveillance campaign was coordinated by CIRAD and Wetlands International, in partnership with national wildlife and veterinary services. The investigations targeted natural sites where waterbirds from various breeding grounds congregate and mix, hence providing the opportunity for AI virus to be transmitted among various host populations and spread over extensive geographical ranges. Study sites were selected in accordance with national surveillance programmes and field logistic constraints. Operations were conducted during 7 to 10-day sampling periods. With the spread of HPAI H5N1 over the TCP region in the course of the survey period, complementary sampling sites were identified in the proximity of recent notified outbreaks (in particular Egypt, Niger, and Burkina Faso; Figure A2.1; Table A2.1).

Target species were selected among bird families recognised as major AI reservoirs (notably Anseriformes and Charadriiformes), in both Eurasian and Afro-tropical bird communities. A restricted number of species were targeted in each study site to maximize the number of samples collected per species.

Table A2.1: Avian influenza surveillance campaign results in Eastern Europe, the Middle East, and Africa in early 2006. (Bird group is indicated only for sample number > 20% total number collected at each sampling site: ED Eurasian Ducks, AD African Ducks, WD Waders, RL Rails, GT Gulls and Terns, CM Cormorants, HS Herons and Storks).

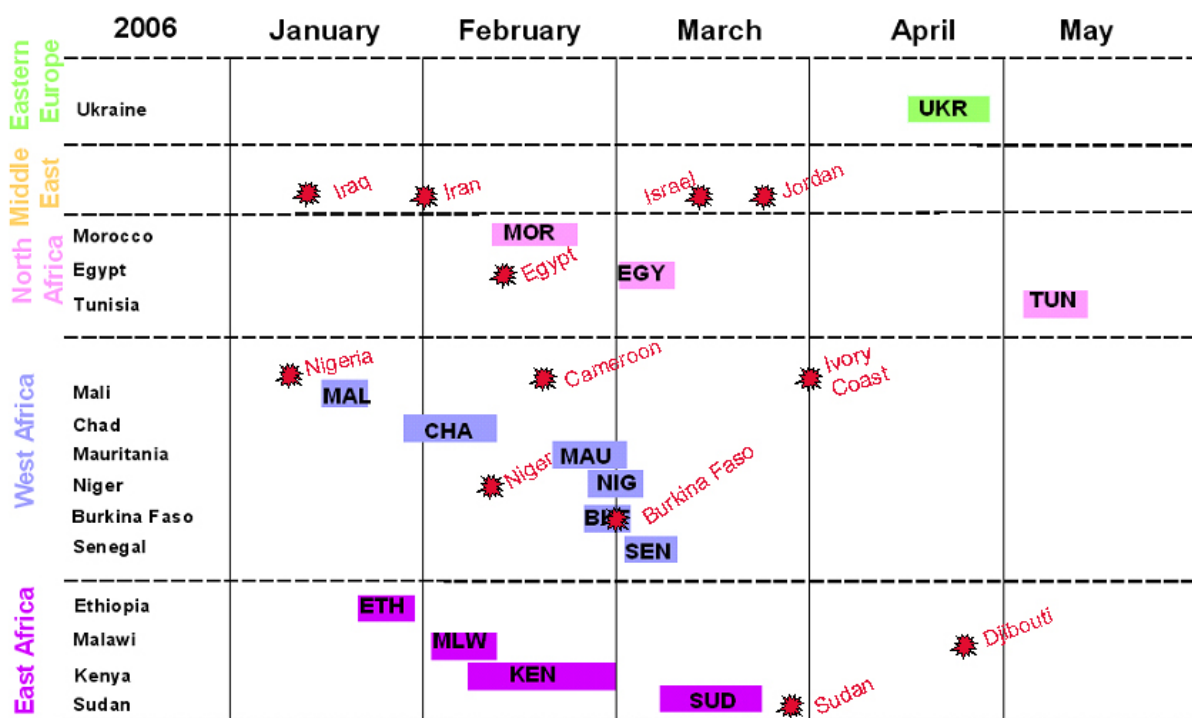
TCP	Area	Country	Sampling site	Period	Samples	Bird Group	Laboratory
Eastern Europe	Black Sea	Ukraine	Sivash / Askania-Nova Biospheric Reserve	April	344	WD	CRL
North Africa	Nile Delta	Egypt	Lake Manzala	March	244	GT, CM	NAMRU3
	Atlantic coast	Morocco	Bas Loukkos marshland	February	91	ED, WD, RL	IZS
	Mediterranean coast	Tunisia	Thyna salt-pans, Sfax	May	51	WD	SEPRL
Western Africa		Chad	Douguia	February	740	ED, AD	IZS
	Lake Chad Basin	Niger	Maradi and Zinder regions	February	98	ED, AD	IZS
	Southern Niger	Niger	Gaya region	March	276	ED, AD	IZS
	South East Burkina Faso	Burkina Faso	Lake Kompienga	February	349	AD, WD	IZS
	Inner Niger Delta	Mali	Mopti region	January	692	ED, WD	IZS
	Senegal Delta	Mauritania	Diawling NP, Lake Aleg	February	462	ED, AD	IZS
		Senegal	Djoudj NP, Langue de Barbarie NP	March	460	ED, AD, GT	IZS
	Atlantic coast	Mauritania	Banc d'Arguin NP	February	279	GT	IZS

Eastern & Southern Africa	Eastern Africa	Ethiopia	Lake Awasa, Debre Zeit, Longano, Ziway	January	115	AD	IZS
	Eastern Africa	Kenya	Dandora Sewage Works, Nairobi	February	286	ED, RL, WD	OVI
	Eastern Africa	Sudan	Am Gar	March	356	WD, HS	Not analysed
	Southern Africa	Malawi	Lake Chilwa	February	413	RL, AD	OVI
Total		14 countries				5,256 samples	

CRL (Community Reference Laboratory, Weybridge, UK); IZS (Istituto Zooprofilattico Sperimentale delle Venezie, Padova, Italia); NAMRU3 (US Naval Medical Research Unit-3, Cairo, Egypt); OVI (Agricultural Research Council Onderstepoort Veterinary Institute, South Africa); SEPRL (Southeast Poultry Research Laboratory, USDA/ARS, USA).

NP National Park

Figure A2.1: Concomitance in timing of field campaigns in surveyed countries (sampling periods distributed along a temporal axis) and first HPAI H5N1 reported outbreaks (OIE-World organisation for animal health notification reports) in the surveyed regions between January and May 2006.



Samples were collected by teams of national and international experts, in collaboration with international conservation and research organizations (AFRING, OMPO-Oiseaux Migrateurs du Paléarctique Occidental, ONCFS-Office National de la Chasse et de la Faune Sauvage, SOVON-Dutch centre for Field Ornithology, WWT-Wildfowl & Wetlands Trust), local ornithological organizations, and national park departments, as well as national hunting associations and safari operators. Cloacal swabs were collected from recently killed birds provided by hunters, and from live-caught birds. In countries with hunting restrictions (Ethiopia) and in countries where emergency surveillance operations were implemented following notification of HPAI H5N1 outbreaks in Nigeria (Burkina Faso, Niger), birds were shot through special permits for sample collection (n= 732). Fresh faecal samples were collected on some occasions at roosting areas for gulls and terns (Laridae) and ducks (Anatidae). Duplicate sampling was performed in the field in order to submit samples to both national and international reference laboratories. The transport medium consisted of an isotonic phosphate buffered saline (PBS), pH 7.0-7.4, containing the antibiotics penicillin (10,000 units/ml), streptomycin (10 mg/ml), amphotericin B (25 µg/ml), and gentamycin (250 µg/ml) supplemented with 10% glycerol. Samples were stored in the field in liquid nitrogen containers or on ice and then stored at < -70°C after a few hours (generally <4h, maximum 24h). They were shipped in dry ice in cryopacks until processed.

Most samples were processed at IZS delle Venezie-Italia, while some samples were analysed in other laboratories (Table A2.1). The samples were all screened by real-time RT-PCR specific for type A influenza viruses (Spackman et al. 2002), and positive samples were then tested by RT-PCR specific for H5 subtype. The molecular pathogenicity of all H5 positive samples was determined by sequencing the haemagglutinin gene segment. Sequences were performed using the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) in a 3100-Avant Genetic Analyzer (Applied Biosystems). On the other

hand, all type A positive samples were subsequently processed for virus isolation using standard methods. Briefly, 100 µl of the original sample were inoculated into the allantoic cavity of 9 to 10-day embryonated specific pathogen free (SPF) eggs for virus isolation attempts according to European Union Directive 92/40. Haemagglutinating isolates were characterized by haemagglutination-inhibition test (HI) and neuraminidase inhibition (NI) test using specific hyperimmune chicken antisera to the reference strains of influenza virus (Alexander and Spackman 1981).

Results

A total of 5,256 samples was collected in 14 countries, mostly on the African continent, and mostly between mid-January and mid-March (Table A2.1, Figure A2.1). Field surveillance operations were postponed in Romania due to severe weather conditions and in Iran and Turkey because of delayed official approval from national authorities.

Samples were collected from 87 bird species, with 17 species representing 90% of all samples collected. A majority of these samples originated from Anatidae, including both Afro-tropical ducks (30% of all samples collected, mostly white-faced whistling duck: *Dendrocygna viduata*) and Eurasian ducks (29%, mostly garganey: *Anas querquedula*). Other species consisted mostly of waders (16%), gulls and terns (11%), rails (9%) and cormorants (3%) (Table A2.2).

Table A2.2: Prevalence of low pathogenic avian influenza virus detected by RT-PCR in wild birds.

Bird group (No. species tested)	No. species	No. tested	No. PCR-Positive (%)	PCR-positive bird species
African ducks	9	1455	41 (2.8)	<i>Dendrocygna viduata</i> <i>Sarkidiornis melanotos</i>
Eurasian ducks	10	1409	93 (6.6)	<i>Anas acuta</i> , <i>A. querquedula</i> , <i>A. crecca</i> , <i>A. clypeata</i>
Eurasian waders	12	688	6 (0.9)	<i>Calidris ferruginea</i> <i>Philomachus pugnax</i> <i>Tringa erythropus</i>
Rails	7	416	3 (0.7)	<i>Gallinula chloropus</i> <i>Porphyrio porphyrio</i>
Gulls	3	366	14 (3.8)	<i>Larus fuscus</i> <i>Larus genei</i>
Terns	3	151	2 (1.3)	<i>Sterna sp.</i> *

* Unidentified fresh faecal samples from a multi-species flock of *Sterna caspia*, *S. maxima*, and *S. sandvicensis*.

The overall prevalence for type A influenza viruses from samples tested by RT-PCR was 3.3% (n=159). No HPAI H5N1 virus was detected, nor any HPAI virus in the samples. Eleven samples were positive for H5 subtype, mostly from garganey (n=10), representing an H5 prevalence of 0.7% in this species.

Low pathogenic avian influenza (LPAI) viruses were detected in 14 species of 5 bird families (Anatidae, Rallidae, Scolopacidae, Laridae, Sternidae), including both Eurasian and Afro-tropical bird species (Table A2.2) from 8 countries (Chad, Ethiopia, Mali, Mauritania, Morocco, Niger, Senegal, and Tunisia). LPAI viruses were detected in Palearctic migratory waterbirds in their over-wintering sites in Africa, including ducks (garganey, northern pintail *Anas acuta*, green-winged teal *A. crecca*, and northern shoveler *A. clypeata*), waders (curlew sandpiper *Calidris ferruginea*, ruff *Philomachus pugnax*, spotted redshank *Tringa erythropus*), gulls (lesser black-backed gull *Larus fuscus*), as well as in some Afro-tropical waterbirds, including ducks (white-faced whistling duck, knob-billed duck *Sarkidiornis melanotos*), gulls (slender-billed gull *Larus genei*) and rails (purple swamphen *Porphyrio porphyrio*). In Anatidae, a higher prevalence was detected in Eurasian ducks (6.5%) than in Afro-tropical ducks (2.8%) (chi-square test, $p < 0.001$).

Five viruses were isolated in embryonated eggs from the 159 RT-PCR positive samples. Three distinct isolates were obtained from garganey in the Inner Niger Delta in Mali, and two isolates were recovered from white-faced whistling duck in Ethiopia and in Senegal (Table A2.3).

Table A2.3: *Virus subtypes isolated from the RT-PCR positive samples.*

Species	Country	Virus isolate
<i>Anas querquedula</i>	Mali	H5N3 LPAI
	Mali	H11N9
	Mali	H12N5
<i>Dendrocygna viduata</i>	Senegal	H1N1
	Ethiopia	H8N4

Discussion

Little information is available about circulation of influenza viruses in waterbirds on the African continent, and the potential for transmission of AI viruses between Eurasia and Africa is poorly understood (Olsen et al. 2006). Our results are the first large-scale AI surveillance in waterbirds over the African continent and beyond.

No evidence was found of HPAI H5N1 virus circulating in wild birds, including samples collected in countries that had experienced recent avian influenza outbreaks, some of which were ongoing at the time of the surveys. However, this absence of H5N1 viruses among thousands of samples investigated must be interpreted in relation to the millions of waterbirds gathering in African wetlands during the northern winter. This outcome is coherent with the absence of H5N1 virus reported from recent surveillance programmes in European countries (EFSA 2006, Pitman et al. 2007) and with the very limited detection rate of H5N1 virus so far from healthy wild bird populations (Chen et al. 2006). However, results from experimental infection in ducks indicate that, contrary to other AI viruses, HPAI H5N1 virus concentration may be higher in the trachea than in the cloaca (Sturm-Ramirez et al. 2004, Hulse-Post et al. 2005), suggesting that HPAI H5N1 virus could have potentially remained undetected in the cloacal and faecal samples we tested.

Avian influenza virus was detected from cloacal swabs and fresh faeces collected from white-faced whistling duck, including samples originating from the same study site (i.e. the Senegal delta). Similar to temperate regions, the collection of freshly deposited faecal droppings can provide a valid method for monitoring LPAI virus presence in tropical regions.

An unusually low virus isolation rate was however obtained from the type A RT-PCR positive samples. Major attention was given to appropriate storage of all samples at $\leq -70^{\circ}\text{C}$, and to the preservation of the cold chain from the field to the lab. Nevertheless, logistic

constrains in some remote field sampling areas and unexpected international shipment delays may account for this low recovery rate.

The measurement of AI virus prevalence in wild birds in Africa provides new insights into the host ecology of AI virus in tropical regions. LPAI viruses were detected in both Palearctic and Afro-tropical waterbirds in several sampling sites, indicating that viruses were circulating in Africa during the northern winter (Appendix Two - Gaidet et al. 2007).

The detection of LPAI viruses in Eurasian ducks in several of their major overwintering sites in West Africa (i.e. Lake Chad, Inner Niger and Senegal River deltas) supports the hypothesis that AI viruses persist in wild duck populations through a continuous circulation in a proportion of birds. The different viruses isolated from Garganey sampled in the Inner Niger Delta in Mali also indicate that various subtypes are circulating at the same time in a single wintering population, in agreement with patterns observed in Europe and North America (Fouchier et al. 2003, Krauss et al. 2004).

The detection of viruses in some Eurasian wader species contrasts with the apparent absence of AI viruses reported in previous studies of waders in Europe (Fouchier et al. 2003), but is consistent with results found in surveillance in North America (Krauss et al. 2004). Several Afro-tropical bird species from various bird families also were found positive for LPAI viruses, raising the possibility of a potential persistence of AI viruses in the tropical environment all year round.

Results from this large-scale surveillance study provide evidence that LPAI viruses circulate in wild birds in sub-tropical environments during the northern winter, including in Eurasian waterbirds wintering in sub-Saharan Africa before their northwards spring migration. This suggests a potential role of tropical regions for the perpetuation of some AI viruses and in their potential intercontinental transmission. At the same time, no evidence was

found for the transmission of HPAI between Eurasia and Africa through bird migration. Such findings stress the need to improve our understanding of the host ecology of AI viruses, in particular in sub-tropical and tropical regions, which should contribute to the prevention and control of HPAI. During fall 2006 and in winter 2007, this surveillance programme implemented within the framework of the TCPs will be replicated and extended over Eastern Europe, the Middle East, and Southern Africa.



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Appendix Three: Bovine tuberculosis in buffaloes, Southern Africa

(Appendix reference: de Garine-Wichatitsky, M., Caron, A., Gomo, C., Foggin, C., Dutlow, K., Lane, E., Le Bel, S., Hofmeyr, M., Hlokwe, T. and Michel, A. 2010. Bovine tuberculosis in buffaloes, Southern Africa. *Emerging Infectious Diseases*, 16 (5) 884-885)



To the Editor: Emerging tuberculosis in Southern African wildlife has implications not only for the conservation of wildlife species affected (Caron et al. 2003), but also for the health of human and livestock living at the wildlife-livestock-human interface (Michel et al. 2006). First diagnosed in African buffaloes (*Syncerus caffer*) in South Africa's Kruger National Park (KNP) in 1990 (Bengis et al. 1996), the disease was likely introduced in the park by cattle-to-buffalo transmission (Michel et al. 2009). Bovine tuberculosis (bTB) infection is spreading northward: in 2003 infection was confirmed in a buffalo approximately 60 km south of the Limpopo river; in 2005 a case was confirmed only 6 km south of the Limpopo (D. Keet, unpub data).

We report the first isolation of *Mycobacterium bovis* from African buffaloes in Zimbabwe. During a survey carried out 9th-13th October 2008, 38 buffaloes from four different herds were captured in Gonarezhou National Park (GNP; south of Mabalauta area; 22.0553°S; 31.4265°E). Blood samples collected from immobilised buffaloes were tested using a gamma interferon assay (Grobler et al. 2002). All sampled buffaloes were marked and released, and three adult females in each group equipped with radio-collars. Four buffaloes (9.5%) tested positive for bTB in the gamma interferon assay, including two adult females and one subadult male from the same herd, and one adult female from another herd. Four months later a collared adult female and the sub-adult male, which had both tested positive on gamma interferon assay, were traced and darted by helicopter. Following euthanasia both animals were necropsied in the field and samples collected from lymph nodes of the head and thorax for histopathology and culture. No acid-fast organisms were detected but the histological findings were strongly suggestive of paucibacillary tuberculosis. *M. bovis* was isolated from the retropharyngeal lymph nodes of both buffaloes and from the bronchial and head lymph nodes of one buffalo. Both isolates were typed by analysis of variable numbers of tandem repeat (VNTR) sequences using six loci (ETR A-F) (Frothingham and Meeker-

O'Connell 1998) and compared with the VNTR profiles of approximately 75 isolates from the KNP. All isolates showed an identical VNTR profile (7544*5 2.3), suggesting an epidemiological link between the *M. bovis* infections in the two parks. However, the ETR loci were shown to have a lower discriminatory power among KNP isolates than IS6110 restriction fragment length polymorphism typing (Hlokwe, unpublished data) (Michel et al. 2009) and a typing regimen comprising different typing methods and markers will be useful to determine the genetic relationship between the isolates from Gonarezhou and KNP more accurately.

The confirmation of bTB infected buffaloes in Zimbabwe GNP raises a number of questions regarding the spread of transboundary animal disease and has considerable management implications for the Great Limpopo Transfrontier Conservation Area (GLTFCA). The most likely scenario is buffalo-to-buffalo contact across the boundary, because the bTB cases reported here were located less than 45 km from the (unfenced) northern boundary of KNP. Buffaloes frequently disperse between herds, especially bulls and young heifers, and may contribute to the spread of *M. bovis* by mixing with naive herds (Cross et al. 2005). Although transboundary movements of buffaloes between KNP and GNP have not been specifically documented, uncontrolled movements across the Limpopo do occur (de Garine-Wichatitsky, personal observation). However, more than 12 wild species have now been demonstrated to be infected by bTB in the KNP (Michel et al. 2006). Most of them are probably not an effective source of *M. bovis* for buffaloes, but bTB epidemiology could rely on multi-host reservoir (Renwick et al. 2007). Thus, a second scenario could involve a buffalo-to-unidentified wild species-to buffalo pathway, as species like greater kudu (*Tragelaphus strepsiceros*) appear to be able to maintain, spread and even drive a bTB epidemic in some cases (Keet et al. 2001, Michel et al. 2009). The third scenario involves movements of infected livestock across the boundaries of the three countries of the GLTFCA,

via a cattle-to-buffalo contamination. As a last scenario, we cannot rule out the possibility that bTB infection of buffaloes has remained silent and undetected for decades in Zimbabwe.

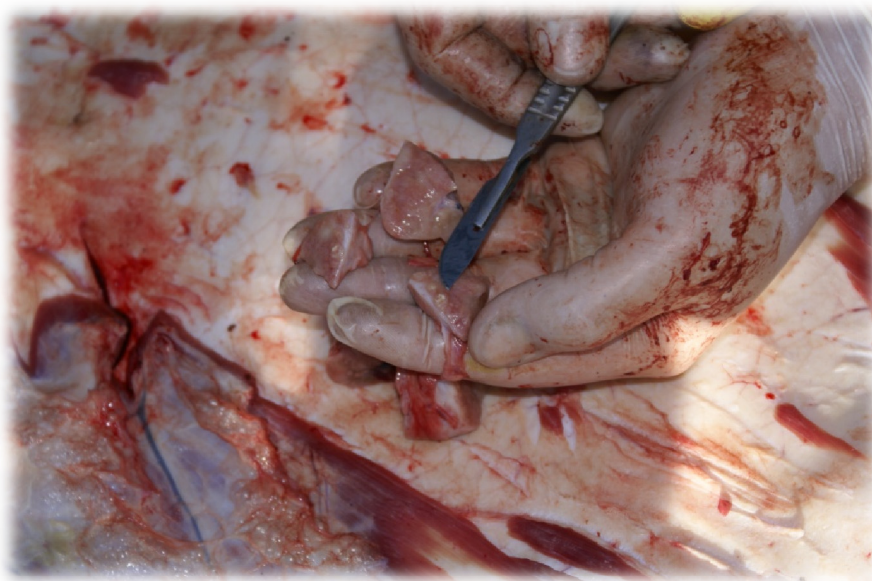
The management implications of the discovery of bTB in buffaloes from GNP are considerable. Once established in a native free-ranging maintenance host, eradication of bTB is unlikely (2,10), and there is an urgent need to evaluate the prevalence and the distribution of the infection in wildlife and livestock populations on the Zimbabwean side of the GLTFCA. Control options of bTB in wildlife are limited (De Lisle et al. 2002, Michel et al. 2006), but chances of success are greater if control measures are initiated at the early stage of the disease spread into a new area. Adequate risk mitigation strategies should also be developed and implemented to reduce the risk of bTB transmission to livestock and humans living at the periphery of the unfenced GNP. Failure to promptly assess the situation and adopt appropriate measures would have far reaching conservation, economic and public health consequences, not only for Zimbabwe, but also for the political and social acceptance of the TFCAs that have been blooming in Southern Africa.



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Appendix Four: Understanding the ecological drivers of avian influenza virus infection in wildfowl: a continental scale study across Africa

(Appendix reference: Gaidet N., Caron A., Cappelle J., Cumming G.S., Balança G., Hammoumi S., Cattoli G., Abolnik C., Serva de Almeida R., Fereidouni S.R., Grosbois V., Tran A., Mundava J., Fofana B., Ould Elmamy B., Ndlovu M., Mondain-Monval J.Y., Triplet P., Hagemeijer W., Karesh, W. B., Newman S.H., Dodman T. 2011. Understanding the ecological drivers of avian influenza virus infection in wildfowl: a continental scale study across Africa. In press in *Proceedings of the Royal Societies Series B*)



Introduction

Infectious zoonoses are a growing concern as the human population expands and contact rates between humans and animals increase (Daszak et al. 2001). A majority of zoonoses are caused by pathogens with a wildlife origin. As we seek to understand and control zoonoses, the influence of host ecology on pathogen transmission is increasingly being recognised as fundamental to the dynamics of wildlife zoonotic disease (Alitzer et al. 2006, Stallknecht 2007, Carver et al. 2009, Tompkins et al. 2010).

Although considerable progress has been made in recent years in linking ecological and epidemiological perspectives, empirical explorations of the interface between ecology and epidemiology are still in an earlier phase (Carver et al. 2009, Tompkins et al. 2010). We currently lack a widely accepted theoretical framework that captures both the ecological determinants of disease transmission and classical epidemiological dynamics. One of the most obvious current barriers to the development of such a framework is the absence of data-heavy empirical tests of hypothesized mechanisms (Carver et al. 2009, Tompkins et al. 2010). Detailed empirical evidence is needed from host population to community levels and across a variety of environmental conditions, scales, and host communities. We address this need through a detailed, continental-scale, empirical analysis of some of the ecological mechanisms underpinning the transmission and perpetuation of avian influenza viruses (AIV) in their main natural reservoir, wildfowl (ducks, swans and geese).

AIV offers an informative case study because it occurs globally with high variations in prevalence in a range of highly mobile and abundant host populations (Olsen et al. 2006), creating both the potential to explore a wide range of environmental influences on transmission and the potential to better understand the role of animal movement and seasonal fluctuations in animal abundance in disease dynamics. The ecology and epidemiology of AIV

have received significantly increased attention in recent years in response to the emergence and spread of the highly pathogenic avian influenza (HPAI) H5N1 viruses across Eurasia and Africa (Hoye et al. 2010), responsible for human fatalities and large economic losses in the poultry industry. Several mechanisms have been proposed whereby host ecology and the environment may influence the dynamics of AIV transmission in wild bird populations (Webster et al. 1992, Krauss et al. 2004, Olsen et al. 2006, Munster et al. 2007, Stallknecht 2007, Munster and Fouchier 2009). However, few empirical investigations of these mechanisms have been conducted in particular across large spatial scales (Hoye et al. 2010).

In what follows, we first present the general ecological factors operating at the host population and community levels and through seasonal environmental drivers, and then provide a detailed analysis of their potential influence on the prevalence of AIV infection measured in a large-scale study of AIV in wildfowl (Figure A4.1). Table A4.1 summarises current understanding of AIV transmission mechanisms in wildfowl.

Although this summary captures many of the basic elements of a general model that links epidemiology and ecology in the context of infectious zoonoses, it is important to note that most of our understanding of AIV infection dynamics is derived from studies that have been conducted in boreal or temperate regions of the northern hemisphere (Krauss et al. 2004, Olsen et al. 2006, Munster et al. 2007). There is a knowledge gap in tropical regions, and in particular in sub-Saharan Africa. Earlier studies have suggested that tropical regions may act as an epicentre contributing to year-round AIV perpetuation in wild birds (Webster et al. 1992). More recently, AIV have been found circulating in various regions of the African continent in both Afro-tropical and migratory Eurasian wildfowl (Appendix Two - Gaidet et al. 2007, Abolnik et al. 2010, Chapter Four - Caron et al. 2011) indicating that local environmental conditions are favourable for the transmission of AIV. The patterns of AIV prevalence observed in temperate or boreal regions cannot be directly transposed to the

tropics where differences in host ecology, climatic constraints and seasonality may produce different dynamics of infection.

In Afro-tropical regions, seasons are determined by rainfall rather than temperatures. The annual migration of the inter-tropical convergence zone (ITCZ) produces a distinct wet season of variable duration according to latitude (Figure A4.1A). The Afro-tropical regions are characterised by high temperatures of relatively low annual variation. During the rainy season many Afro-tropical wetlands experience extreme seasonal variations in their surface area (Conway et al. 2009). Rivers may swell rapidly, after a relatively short but intense rainy season, with the capacity to inundate vast floodplains. Many wetlands are ephemeral, due to the long dry season and high evaporation rates. At the end of the dry season, water bodies are generally limited to a few permanent wetlands where waterbirds congregate.

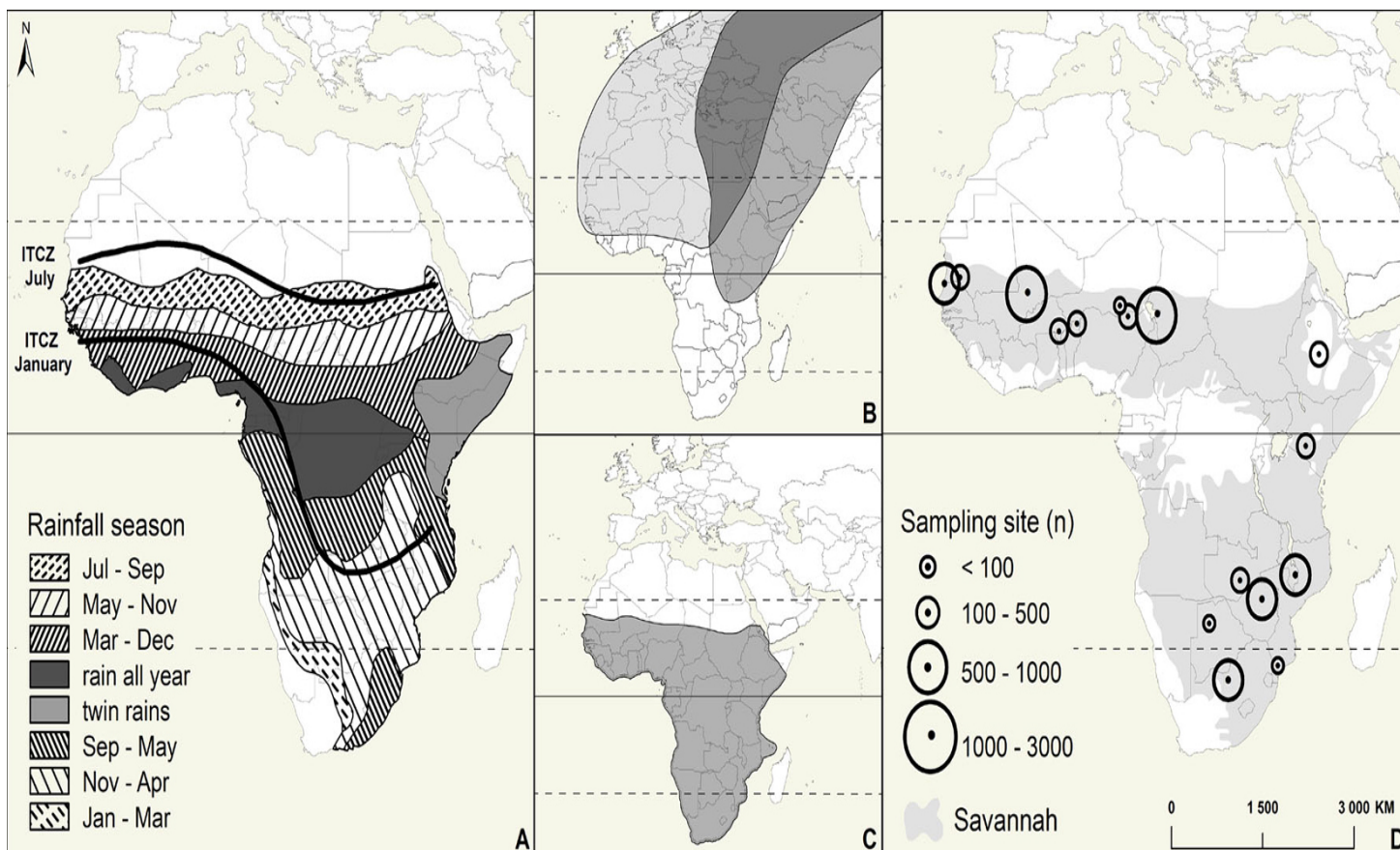
Sub-Saharan Africa north of the equator constitutes a seasonal non-breeding area for a large number of Eurasian (i.e. Palaearctic-breeding) migratory ducks between September and March (Zwarts et al. 2009) (Figure A4.1B), including eight widespread species and four uncommon species (Wetlands International 2006). Most are dabbling ducks of the *Anas* genus, the most numerous being Garganey (*A. querquedula*) with populations of c. 2 million birds (Scott and Rose 1996). All African regions, including those south of the equator, are also connected to Eurasia by other Eurasian migratory waterbirds including waders, gulls, terns, rails, herons and storks.

Table A4.1: Summary of our current understanding of AIV transmission in wildfowl in relation to host ecology and environmental drivers.

Epidemiological parameters	Experimental ¹ or empirical ² evidence and epidemiological predictions ³	Sources of ecological variations	Ref.
Environmental transmission	¹ AIV can remain infectious for several months in water under experimental conditions. Warmer temperatures, radiation and desiccation reduce the duration of AIV infectivity	Climatic constraints on the persistence of AIV in the environment	8,12
	² Mathematical water-borne transmission models captured some patterns of AIV infection dynamics in wildfowl		13,14
	¹ Successful experimental infection of ducks by contact with contaminated water		15
	² Higher prevalence in dabbling ducks (foraging mainly in surface water) than in diving ducks (feeding in deeper water) or grazing wildfowl (foraging on grasslands)	Species foraging behaviour affecting host exposure to environmental infection	6,11
Inter-individual transmission	³ Density-dependent transmission may occur if the contact rate between susceptible and infected birds increases with host density	Host density and seasonal congregation affecting contact rate	16
	² The northern autumn peak in AIV prevalence in ducks coincides with bird seasonal flocking at pre-migration and stopover staging are		8,9,17
Transmissibility	³ Potential intrinsic differences in permissiveness to AIV infection between wildfowl species accounted by differences in distribution of virus receptor types	Species evolutionary history	8,11
	² Higher prevalence consistently reported in <i>Anas</i> compared to other wildfowl species		6,10
Host susceptibility	³ Immunological naivety of birds with no previous exposure to AIV increase their susceptibility to infection	Host age and species social or migratory behaviour influence on previous AIV exposure	8,11,18
	¹ Evidence of cross-protective immunity against AIV re-infection		19
	¹ Age at infection affects the extent of viral shedding in experimentally infected wildfowl		20
	² A higher AIV prevalence in hatch-year birds compared to after-hatch-year birds is consistently reported		10, 21
Herd immunity	³ The proportion of susceptible individuals in the host population may control disease transmission rate	Demographic rate and migration influx affecting turnover of susceptible hosts	2
	² Proportion of hatch-year bird in wildfowl populations gradually decreased along the flyway during the autumn postnuptial migration		22
	² Prevalence decline during Northern autumn and winter as the proportion of naïve birds progressively decreases through infection or death	Timing of reproduction and congregation influence seasonal fluctuation in population immunity	9,23, 10,17
Host dispersal	¹ Experimentally infected wildfowl generally excrete a high concentration of AIV for 1-3 weeks without apparent signs of disease	Range and timing of host migration depending on species	19,20

² Migratory wildfowl are able to perform long-distance movements within the time frame of AIV infection	and breeding regions	24
² Phylogenetic analysis confirms the occurrence of inter-continental exchange of viruses via migratory wildfowl		25
² Phylogeographic analysis suggest a dominance of migration over persistence process in the interannual perpetuation of AIV in wildfowl		26

Figure A4.1: A. The timing and duration of rainfall in sub-Saharan Africa and the seasonal position of the Inter-Tropical Convergence Zone (ITCZ) (30); B. The two main migratory flyways of Eurasian wildfowl wintering in sub-Saharan Africa; C. Distribution range of Afro-tropical wildfowl over the African continent; D. Location of sampling sites presented in our study, also showing sample size ranges.



In their wintering sites, Eurasian ducks congregate and mix with Afro-tropical wildfowl that reside year-round within sub-Saharan Africa (Figure A4.1C). The diversity of Afro-tropical wildfowl (31 species) is similar to that of Eurasian wildfowl (39 species), but only a few species ($n=3$) have a population in excess of 500,000 birds (Wetlands International 2006). Several Afro-tropical wildfowl species are widespread over sub-Saharan Africa, some of which are at least partially migratory, including trans-equatorial movements (Scott and Rose 1996). Breeding generally occurs during or following the wet season, but Afro-tropical wildfowl have extended breeding seasons compared to Eurasian wildfowl, with laying periods stretching over 6 to 12 months (Brown et al. 1982).

Given the many differences between temperate and tropical environments, it is unclear how well the elements of a general framework presented in Table A4.1 capture key dynamics in Afro-tropical ecosystems. To address this question, and to extend our empirical knowledge of AIV, we undertook a large-scale analysis of data on AIV in free-living wildfowl that were collected from 15 African countries (Figure A4.1D, Supplementary Information (SI) Table A4.S1) during 2006-2009. We analysed host, seasonal and geographical variations in AIV prevalence and focused on two potential but non-exclusive processes that potentially control the dynamics of AIV transmission in Afro-tropical regions: a seasonal introduction and spillover of AIV from Eurasian migratory waterbirds, and an endemic cycle (i.e. a year-round perpetuation of AIV by wildfowl in Afro-tropical ecosystems).

Our analysis is structured around six predictions. If Eurasian migratory waterbirds, and in particular Eurasian wildfowl, are the prime source of introduction of AIV in Afro-tropical regions, we predict that local and seasonal AIV prevalence should be related to: i) the geographic origin of species sampled, with a higher prevalence in Eurasian than in Afro-tropical wildfowl; ii) the proportion of Eurasian wildfowl species in the local wildfowl community; and iii) the timing of arrival of Eurasian migratory waterbirds, with a seasonal

peak in prevalence when migrants arrive followed by a decrease as the level of population immunity increases.

In addition, in the case of an endemic AIV cycle in Afro-tropical regions, we predicted iv) a higher prevalence in *Anas* than non-*Anas* species irrespective of their geographic origin. We also expected transmission of AIV to be driven by ecological factors homologous to those proposed for temperate and boreal regions (Webster et al. 1992, Krauss et al. 2004, Olsen et al. 2006, Munster et al. 2007, Munster et al. 2009) but adapted to the ecological context of Afro-tropical ecosystems. If climatic constraints limit the survival of AIV in the environment in the tropics, we predicted that v) AIV prevalence would be influenced by ecological factors associated with density-dependent transmission (host density and seasonal congregation) rather than with environmental transmission (climatic indices and species foraging behaviour). Lastly, since Afro-tropical regions are characterised by a more gradual rate of recruitment of susceptible juveniles (due to extended breeding seasons), and since seasonal congregation of wildfowl is related to the progressive reduction in the surface of wetlands during the dry season rather than to flocking before and during migration, we predicted that vi) the seasonality of AIV transmission will increase progressively from the onset of the wet season to the end of the dry season in response to both a progressive supply of susceptible hosts and a progressive congregation of wildfowl at permanent water bodies during the dry season.

Materials and Methods

Samples originated from apparently healthy live-caught birds captured using mist-nets and baited walk-in traps, or from freshly killed birds provided by hunters. Samples were collected throughout the year (with at least two months between sampling occasions). Birds

were tested for AIV from three types of samples that we distinguished in our analyses: double (cloacal + oropharyngeal swabs tested individually), single cloacal or single oropharyngeal. Samples were analysed in different laboratories using a similar standard diagnostic procedure based on RNA extraction and real-time RT-PCR virus detection (see SI Methods for a complete descriptions of sampling and diagnostic methods). We estimated prevalence for each species, sampling site and sampling occasion, as the percentage of individuals found positive for AIV as compared with the total number of birds tested.

Ground-based and satellite-based data were used to estimate explanatory variables used to quantify the ecological factors considered in our analysis (see SI Methods). We used a generalized linear modeling approach to relate ecological factors to the prevalence of AIV in wildfowl. A beta-binomial distribution with a logit link function (function `betabin` in the R-package `aod`) was applied to account for the aggregations of positive birds within our samples. We then used an information-theoretic procedure based on the Akaike information criterion corrected for small sample sizes (AIC_c) to select the most parsimonious model from among a set of candidate models with different combinations and numbers of variables (Burnham & Anderson 2002). We ranked models using ΔAIC_c , i.e. the difference of AIC_c values between a given model and the model with the lowest AIC_c values in the set of models. We also computed Akaike weight (ω_i) for each model i that gives the relative likelihood of a given model to be the best among a set of models fitted. We estimated the relative importance of each explanatory variable by summing the Akaike weights ($\Sigma \omega_i$) of all models in the set where that variable occurred. We tested for a quadratic relationship for variables whose influence was predicted to show a cyclical annual variation (time/ arrival Eurasian migrants and time/onset the wet season). Since inter-annual variations in AIV prevalence are commonly reported for wildfowl (Krauss et al. 2004, Munster et al. 2007), we controlled for

the potential confounding influence of individual years in our analyses, as well as for two other ‘nuisance’ parameters (laboratory and sample type).

Our approach consisted of three steps. First, we determined the core variables for each factor presenting alternative explanatory variables (Table A4.S4), testing successively each variable by permutation in a set of global models. Second, we evaluated the relative importance of each ecological factor by building a set of candidate models representing all possible linear combinations of the core variables, testing non-independent variables successively by permutation. Finally, we computed intercepts and model coefficients through model averaging across all models having $\Delta AIC_c < 2$, weighting parameter estimates for each model by the model’s Akaike weight and summing the weighted estimates (Burnham & Anderson 2002).

Results

We sampled and AIV-tested a total of 8414 free-living wildfowl of 19 species, of both Eurasian (32%) and Afro-tropical (68%) origin (Table A4.S2). AIV were detected by RRT-PCR in 278 birds (3.3%, 95% confidence interval (CI) 2.9-3.7%). AIV were detected in almost all countries and in all species for which more than 31 birds were sampled. Prevalence was highly variable between species, sites and sampling occasions, reaching up to 14.7% (CI 10.6-19.9%) in Garganey in Mauritania in February 2006. Detailed results of AIV subtypes detected and isolated are presented in Table S3.

The study sites varied greatly in abundance and composition of the local wildfowl community as well as climatic conditions (Table A4.S1). Local wildfowl abundance ranged from a few thousand birds to about a million birds, with densities reaching up to 1500

birds/km². The proportion of Eurasian wildfowl varied between 0-97% according to sites and seasons. The study sites stretched over four aridity classes (from arid to humid), with mean and maximum annual temperatures ranging between 19–28°C and 26–36°C respectively.

We tested the relative influence of six ecological factors on AIV prevalence, including climatic conditions, species traits, host density, influx of Eurasian wildfowl, timing relative to seasonal congregation and to arrival of Eurasian migrants, as well as three potential nuisance parameters (see *Materials and Methods*). A list of variables associated with each ecological factor tested in our study is summarized in Table A4.S4.

The initial selection among alternative variables associated with the same ecological factor, and tested successively by permutation, indicated that the best predictors of AIV prevalence were: annual PET ($\Sigma\omega_i=0.39$) compared to the six other climatic variables, wildfowl community density ($\Sigma\omega_i=0.92$) compared to sampled species density ($\Sigma\omega_i=0.08$), and species taxonomy ($\Sigma\omega_i=0.60$) compared to geographic origin ($\Sigma\omega_i=0.36$) and foraging behaviour ($\Sigma\omega_i=0.04$) (Table A4.S5).

The AIC-based selection procedure of the complete set of models indicated that four of the six ecological factors tested were important to explain the variation of AIV prevalence in wildfowl (Table A4.S6). The high Akaike importance weights of species taxonomy ($\Sigma\omega_i$ of the models with this factor=0.97), wildfowl community density ($\Sigma\omega_i=0.98$) and the timing relative to arrival of Eurasian migrants ($\Sigma\omega_i=0.89$) indicate that they occurred in a majority of high ranking models. Seasonal congregation of wildfowl estimated from the timing relative to onset of the wet season ($\Sigma\omega_i=0.60$) was also relatively well supported as an important variable across all models. Inclusion of variables associated with the influx of Eurasian wildfowl (% Eurasian wildfowl species, $\Sigma\omega_i=0.35$) or climatic conditions (annual PET, $\Sigma\omega_i=0.44$) received

much less support from the data. Finally, controlling for year ($\Sigma\omega_i=0.91$) and (to a lesser extent) for sample type ($\Sigma\omega_i=0.54$) was important.

Across the full set of models, the best supported overall model ($AIC_c=401.9$, $\Delta AIC_c=0$) was one that included all four of these factors, as well as the two nuisance parameters year and sample type (Table A4.S7). This model fitted the data adequately (lack-of-fit test $P=0.85$). Variations in the level of infection (i.e., in observed prevalences) were relatively well predicted by this model, but the absence of infection (i.e. null prevalences) were poorly predicted (Figure A4.S1). This is likely related to detection limit associated with small sample size in some sampling occasions: infected birds may have been present, but at prevalence below the level of detection of the study (Hoye et al. 2010).

All the other five models that received substantial support from the data ($\Delta AIC_c<2$) included the factors of species taxonomy, wildfowl community density and timing relative to arrival of Eurasian migrants, as well as the timing relative to the onset of wet season for a majority of these models (Table A4.S7). Some of these models also contained factors of low relative importance (% Eurasian wildfowl species, annual PET) as seen by their low Akaike weight across all models.

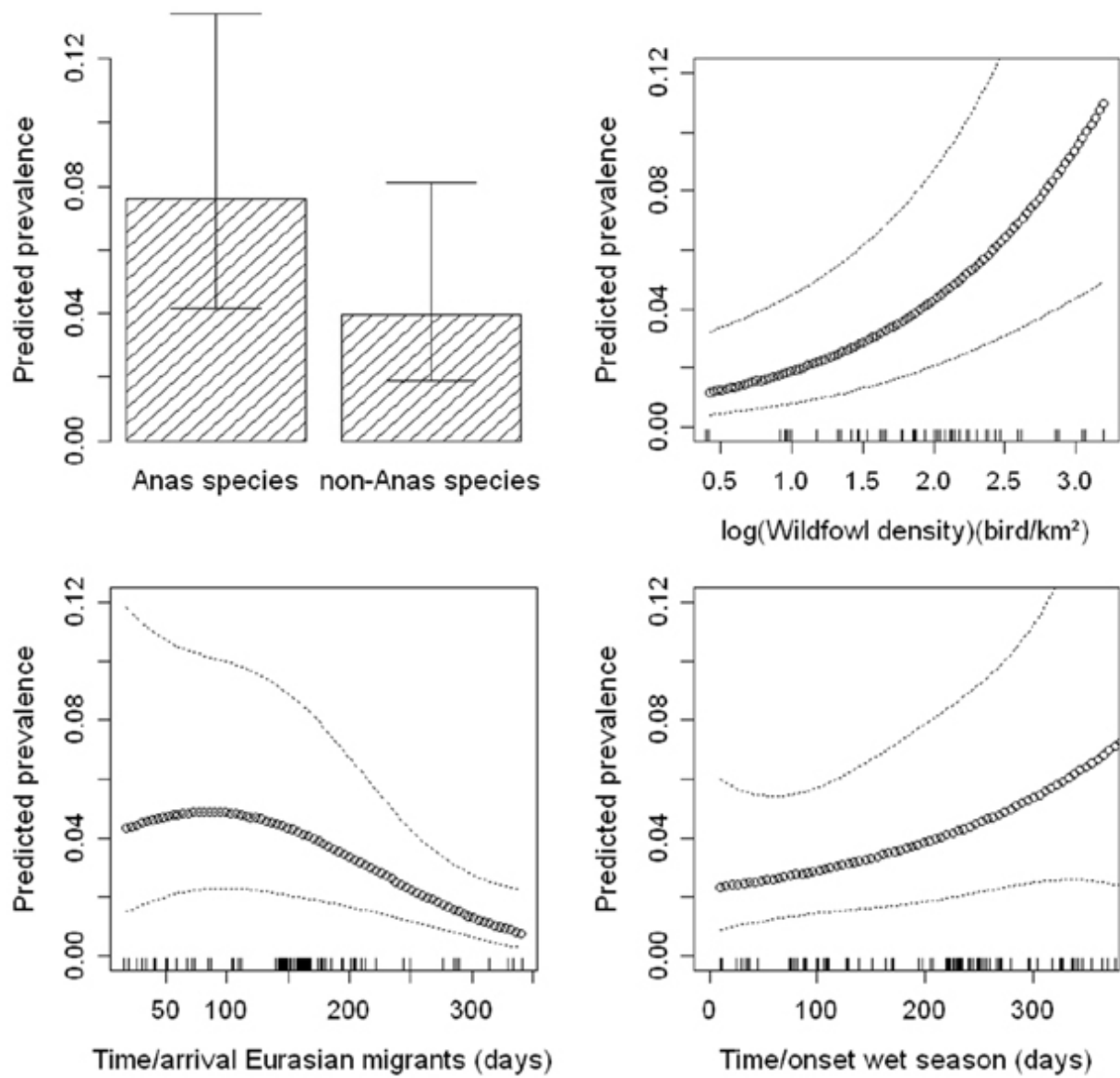
The coefficients of the parameter estimates averaged from the six top models are given in Table A4.S8. AIV prevalence was higher in *Anas* species than in non-*Anas* species and positively associated with the density of the wildfowl community (Figure A4.2). Seasonal variations in AIV prevalence were related to timing relative to both the arrival of Eurasian migrants and the onset of the wet season: prevalence was high and slightly increased during the first half of the wintering period of Eurasian migrants, after which it decreased. Prevalence also progressively increased through the wet season and into the dry season, peaking in the late dry season.

Discussion

Our results show that at a continental scale, the prevalence of AIV infection in wildfowl is strongly influenced by several ecological factors. These factors operate at different levels of ecological organization (host population, community and species) and through environmental drivers. In addition, our results strongly support the hypothesis of an endemic cycle of AIV in Afro-tropical ecosystems rather than a seasonal introduction by Eurasian migratory waterbirds and spillover to African wildfowl. The overall prevalence of AIV found in this study (3.3%) is comparable to AIV detection rates reported in other large-scale studies using similar RRT-PCR screening methods (Munster et al. 2007). AIV were found circulating in all Afro-tropical regions, in all seasons and in all wildfowl species for which a significant number of birds had been tested (Table A4.S2).

In accordance with our initial predictions, our findings suggest that the ecological factors influencing AIV transmission in Afro-tropical regions are derived from specific environmental constraints. The influence of the seasonal congregation of wildfowl on AIV prevalence in the tropics results from the seasonal dynamics of wetland availability instead of the seasonal migration flocking that is typically observed in boreal and temperate regions (Webster et al. 1992, Krauss et al. 2004, Munster et al. 2007). The intensity of such congregation process will vary according to site-specific environments, but could be locally of great importance. For instance, in the Inner Niger Delta in Mali, seasonally flooded wetlands may be up to twenty times as large as the surface area of permanent wetlands (Conway et al. 2009). The increase in local wildfowl density at permanent wetlands in response to the seasonal drying of wetlands likely promotes contact among birds, hence transmission. The positive association that we found between the local wildfowl density and AIV prevalence between sites also supported the hypothesis of a density-dependent transmission process.

Figure A4.2: Effect plots of the four ecological factors identified as influencing the variation of AIV prevalence in wildfowl in Afro-tropical regions. Plots of predicted prevalence (95% CI) were generated from the highest rank model presented in Table A4.S7. Data points are plotted as rug plots.



We found no evidence for an influence of climatic constraints on AIV prevalence. Afro-tropical regions are characterised by mean monthly temperatures $>20^{\circ}\text{C}$ in all months (except in African highlands). By contrast, boreal and temperate regions are characterised by mean monthly temperatures $<20^{\circ}\text{C}$ during at least 8 months per year. Temperatures in most Afro-tropical wetlands may be over a threshold where high temperatures throughout the year limit the duration of virus persistence in the environment. In such a context, the environment may have only a minimal role as a source of infection. In African highlands, however, the existence of a cold season may be compatible with the longer-term persistence of viruses in the environment.

Foraging in surface water, typical amongst dabbling wildfowl, or morphological traits associated with filtration of food particles, have been proposed to promote environmental transmission in wildfowl (Olsen et al. 2006, Munster et al. 2009, Hill et al. 2010). In our study, foraging behaviour was a poor predictor of species variation in prevalence. This finding is consistent with a predominant bird to bird transmission of AIV in the tropics rather than a water-borne transmission.

Our findings indicate that the dynamics of AIV infection in wildfowl in Afro-tropical regions are driven by a density-dependent rather than an environmental transmission process. In temperate regions, by contrast, mathematical models suggest a greater role for indirect transmission of AIV from an environmental reservoir than for density-dependent direct transmission (Roche et al. 2009, Rohani et al. 2009). Such differences in the predominant routes of transmission between temperate and tropical zones have also been suggested for human influenza viruses (Lowen and Palese 2009), in response to specific climatic constraints in the tropics. Since the aerosol transmission of human influenza viruses is highly sensitive to humidity and temperature, transmission is considered to occur predominantly by an aerosol

route during the winter in temperate regions, and mainly through direct or indirect year-round contact in the tropics.

Interestingly, most of our initial predictions associated with a seasonal introduction and a spillover of AIV from Eurasian migratory waterbirds were not supported by our data. Species taxonomy (*Anas* versus non-*Anas* species) was a better predictor of species variation in prevalence than geographic origin. Overall, *Anas* species had higher prevalence than non *Anas* species (Figure A4.2), but the prevalence rates of Eurasian *Anas* species (4.9%, CI 4.2-5.8%) and African *Anas* species (5.2%, 4.1-6.5%) were similar (proportion test, $\chi^2_1=0.11$, $n=4005$, $p>0.3$). An ancestral co-evolution between AIV and their main host, i.e. *Anas* species, may have resulted in more efficient virus binding and replication in these species (Webster et al. 1992, Munster et al. 2009). In addition, variations in AIV prevalence were poorly related to the percentage of Eurasian wildfowl in the local wildfowl community and AIV was found circulating in seasons and regions when and where Eurasian wildfowl were either abundant or absent.

Several authors have proposed the existence of a north-south decline of AIV prevalence in migratory wildfowl, resulting from a progressive increase in the level of population immunity as birds migrate southward in autumn (Webster et al. 1992, Krauss et al. 2004, Guberti et al. 2007, Munster et al. 2007). A strong seasonal decline in prevalence is commonly observed in wildfowl during autumn in boreal and temperate regions, up to a basal level during northern winter months (generally $\leq 1\%$; Webster et al. 1992, Munster et al. 2007). By contrast, we found a relatively high prevalence in Eurasian wildfowl from January to March in sub-Saharan Africa (4.9%, CI 4.2-5.8%) with no seasonal decline during their wintering period. This pattern suggests that the level of population immunity in Eurasian wildfowl may remain relatively low throughout the wintering period in sub-Saharan Africa.

The most abundant Eurasian wildfowl wintering in sub-Saharan Africa, Garganey, may have a low exposure to previous AIV infection before arriving in Africa. This species is an earlier and longer-distance migrant than other Eurasian wildfowl (Scott and Rose 1996), with a substantial passage over North Africa in August and September (Zwarts et al. 2009). Moreover, Garganey usually moult in small numbers and do not form large flocks during migration (Zwarts et al. 2009). Garganey may thus escape the major peak of AIV transmission experienced by other wildfowl in autumn in Eurasia. The influence of such migratory behaviour on AIV prevalence has been proposed for another early migrant in North America, the Blue-winged Teal *Anas discors*, for which high prevalence (c. 10-15%) has been consistently reported during winter (Hanson et al. 2005, Ramey et al. 2010).

The prevalence of AIV in wildfowl in our data was higher during the half of the year when Eurasian migrants winter in Afro-tropical regions (c. September-March) than when they were absent from the continent. The presence of Eurasian migrants may influence the local dynamics of AIV transmission in different ways in regions north and south of the equator. In the northern Afro-tropical regions, the arrival of Eurasian wildfowl constitutes a drastic increase in local wildfowl abundance but may also provide a large population of susceptible birds if they have escaped previous AIV infections. Once on their African wintering grounds, Garganeys are highly gregarious. The first months of their wintering period however coincide with the end of the wet season when the area of wetlands is maximal. Eurasian and Afro-tropical wildfowl progressively congregate in shared wetlands as wetlands dry down. Prevalence may thus remain relatively constant throughout the wintering period. Eurasian wildfowl probably leave Africa before an increase in their population immunity reduces transmission rate and prevalence. High AIV prevalence was accordingly found on several occasions in Garganey at the end of their wintering period (February and March) in West

Africa (14.7%, CI 10.6-19.9% in Lake Aleg, Mauritania in 2006; 14.0%, CI 9.0-21.4%, and 15.1%, CI 7.6-27.1%, in the Senegal Delta in 2006 and 2008 respectively).

Alternatively, in African wintering grounds, Eurasian wildfowl may be susceptible to subtypes distinct from those that predominated during their migration across Eurasia (Hanson et al. 2005). Only a few viruses were characterised during our study preventing exploration of this assumption. It is however notable that the AIV subtypes isolated in Afro-tropical regions do not belong to the most common subtypes reported in wildfowl in Europe (Munster et al. 2007).

Eurasian wildfowl do not generally winter in the southern Afro-tropical region, where host species of Eurasian origin are limited to other migratory waterbirds, including shorebirds. A much lower prevalence has been reported globally in non-wildfowl species ($\leq 2\%$, Olsen et al. 2006, Krauss et al. 2010) suggesting that they play a minor role in the perpetuation of AIV, though locally shorebirds may have a significant role (Krauss et al. 2004, Krauss et al. 2010). Phylogenetic analyses of AIV isolated from wild birds worldwide indicate that inter-continental transfer of AIV genes occurs more frequently in shorebirds than in wildfowl (Dugan et al. 2008). AIV isolated in wildfowl in Southern Africa contained genes whose closest relatives were in viruses found in Europe and Asia, suggesting that migratory shorebirds may constitute a source of AIV introduction in this region (Abolnik et al. 2010).

The predominant role of density-dependent transmission in Afro-tropical regions suggested by our results implies that AIV should be maintained year round through a continuous circulation among wild birds. Maintenance should occur at a relatively low prevalence throughout the year to slow down the controlling effect of herd immunity. In the Afro-tropical context, the ecological factors positively associated with AIV prevalence have a slow seasonal dynamic. The seasonal congregation of wildfowl results from a progressive

gathering of birds throughout the dry season, and the extended breeding seasons result in a low turnover of young susceptible birds in host populations. In accordance, the seasonality of influenza infection we measured in our study was less pronounced (seasonal peak in prevalence $\leq 15\%$) than in Europe (25%, Wallensten et al. 2007) or North America (40%, Krauss et al. 2004). Analogous differences in human influenza seasonal patterns between temperate and tropical regions have also been reported, with a low background influenza activity throughout the year in the tropics compared to high seasonal epidemics in temperate countries (Lowen and Palese 2009).

Regional differences in the composition of the wildfowl community may also determine the background level at which AIV are perpetuated in tropical Africa. *Anas* species likely play a major role among wildfowl in the perpetuation of AIV, as their consistently higher prevalence suggests (Olsen et al. 2006, Munster et al. 2007). These species are, however, not homogeneously distributed among Afro-tropical regions. In West Africa, *Anas* species are represented almost only by Eurasian migratory ducks (Scott and Rose 1996), and therefore there are almost no *Anas* ducks during c. 6 months of each year. African *Anas* ducks are abundant year round in both Eastern and Southern Africa, but Eurasian *Anas* ducks are largely absent in the regions south of the equator. As a consequence, AIV may be perpetuated annually at a background level that decreases sequentially between Eastern, Southern and West Africa.

AIV may be perpetuated at a continental scale through a meta-population process between Afro-tropical regions. There is an asynchrony in the timing of rainfall and associated seasonal ecological drivers influencing AIV transmission between regions north and south of the equator. This may create a network of complementary areas across the Afro-tropical regions with temporarily suitable conditions, in terms of host density and production of susceptible juvenile birds, for AIV maintenance. Local declines or extinction in AIV

circulation may be balanced by a seasonal re-introduction through exchanges of host populations and AIV dispersal within regions (Chen and Holmes 2009). Waterbirds in Africa make a wide range of movements, largely based on rainfall patterns and including some nomadic movements. However, the extent and frequency of intra-African waterbird migrations remain poorly understood, limiting our abilities to predict the level of interaction between regional AIV cycles.

Finally, our results support the hypothesis that Afro-tropical regions may contribute to the global year round perpetuation of AIV by providing a seasonal shelter for the maintenance of AIV in wildfowl (Webster et al. 1992). With prevalence remaining relatively high in sub-Saharan Africa throughout the northern winter period, Eurasian migratory waterbirds may re-introduce some AIV into temperate and boreal regions during their spring migration. In our study, some Garganey were found infected at high prevalence at the time they leave their Sahelian wintering areas (February-March), but also during spring migration in North Africa as they return to their Eurasian breeding grounds (5.7%, CI 1.2-18.6%, in Egypt in April, Table A4.S9). Up to now, however, no African lineage of AIV has been reported in birds in Europe, though few African viruses have been characterised.

This study demonstrates the value of integrating ecology and epidemiology for understanding complex multi-host epidemiological systems (Stallknecht 2007). Our results also demonstrate the importance of obtaining detailed data across a wide range of environmental conditions and host communities (Tompkins et al. 2010). In the development of general models of avian influenza dynamics, it is clear that some mechanisms (e.g. environmental transmission) may have been over-emphasized in the peer-reviewed literature, while others (e.g. responses to seasonal fluctuations in habitat) have been largely ignored because their influence is less obvious in temperate regions. As our analysis shows, research

at the interface between ecology and epidemiology could benefit hugely from inter-group data sharing and detailed empirical analyses of geographically diverse data sets.



Supporting Information (not displayed in the thesis)

Additional Supporting Information may be found in the online version of this article:

Table A4.S1: List of sites ranked by latitude where wildfowl were sampled during this study. Estimates of abundance and composition of the local wildfowl community at the time of sampling are presented for each site, as well as the mean annual climatic conditions. Range values (min-max) are presented for sites sampled on several occasions. These data represent only values estimated for the periods when sampling was conducted (2006-2009), and more extreme values occur.

Table A4.S2: Prevalence of avian influenza viruses in Eurasian (a) and Afro-tropical (b) wildfowl species sampled in this study and tested by real-time RT-PCR.

Table A4.S3: Table S3. List of AIV subtypes a) detected by conventional RT-PCR specific for H5 or H7, or b) isolated in embryonated SPF chicken eggs.

Table A4.S4: Definition of the explanatory variables associated with the ecological factors examined in our analyses.

Table A4.S5: Selection of alternative explanatory variables associated with the same ecological factor, tested successively by permutation in a set of global models. Bold text depicts the selected explanatory variables based on their relative importance, estimated by summing the normalized Akaike weights ($\sum \omega_i$) over the subset of model which contained that variable.

Table A4.S6: Relative importance of selected explanatory variables explaining variations in AIV prevalence in wildfowl in Afro-tropical regions estimated by summing their Akaike weights ($\sum \omega_i$) over the subset of models that contain that variable.

Table A4.S7: Selection statistics of the top ten candidate beta-binomial models describing the variations in AIV prevalence in wildfowl in Afro-tropical regions. Models are ordered by AIC_c rank and the six best-supported models ($\Delta\text{AIC}_c < 2$) are highlighted in bold.

Table A4.S8: Model-averaged parameter estimates of the relationships between AIV prevalence (logit) in wildfowl and four ecological factors and two nuisance parameters identified as important predictors.

Table A4.S9: Prevalence of avian influenza viruses in Eurasian wildfowl sampled in one additional sampling site in North Africa (Nil delta, Egypt) in April 2008.



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Appendix Five: The ecology of Influenza A viruses in wild birds in southern Africa

(Appendix reference: Cumming, G. S., Caron, A., Abolnik, C., Catolli, G., L.W. Bruinzeel, C.E. Burger, K. Cecchetti, N. Chiweshe, B. Mochotlhoane, G.L. Mutumi, M. Ndlovu. 2011. The ecology of Influenza A viruses in wild birds in southern Africa. *EcoHealth* 8(1): 4-13)



Introduction

Influenza A viruses have long been acknowledged as pathogens of global concern. In recent years, outbreaks of highly pathogenic avian influenza (HPAI) in populations of domestic and wild birds, and the related deaths of nearly 300 people (WHO 2010), have heightened fears of a new influenza pandemic in the human population (e.g. Enserink 2006, Pickles 2006). Assessment of the risks that are posed by avian influenza, and the development of appropriate response strategies in the event of an epidemic or pandemic, rely heavily on a fundamental scientific understanding of Avian Influenza Virus (AIV) dynamics in populations of domestic and wild birds (Dudley 2008).

Although low pathogenicity avian influenza (LPAI) viral prevalence in Western European and North American populations has been well documented (Olsen et al. 2006), it is unclear how the long-distance movements of migratory and nomadic bird species relate to larger-scale spatial and temporal variation in AIV recombination, maintenance, and epidemics (Kilpatrick et al. 2006, Krauss and Webster 2010). One of the largest single gaps in the geographical coverage of AIV sampling has been southern Africa (Kilpatrick et al. 2006, Olsen et al. 2006, Appendix Two - Gaidet et al. 2007), a region that is at risk following the detection of highly pathogenic strains in sub-Saharan Africa north of the Zambezi (Gaidet et al. 2008, Fasina et al. 2009). Although some intriguing data exist from South Africa (e.g. Sinclair et al. 2005, Abolnik et al. 2009, Abolnik et al. 2010), little relevant research has been carried out in most southern African countries.

By comparison to western Europe, southern Africa has a relatively mild winter; highly variable and often scarce rainfall; a higher diversity of bird species; no true geese or swans; and many nomadic waterbirds but no truly migratory afrotropical *Anas* ducks (Underhill et al. 1999, Cumming et al. 2008). We tested the predictions that (1) due to its more arid

environment and absence of migratory palearctic ducks, LPAI prevalence in wild waterbirds should be lower in southern Africa than in Europe; (2) due to the presence of many opportunistic, colonial, and nomadic waterbird species, and the lack of migratory corridors (Hockey 2000), LPAI prevalence in wild birds in southern Africa should show relatively little spatial variation along longitudinal and latitudinal gradients; and (3) the arrival of palearctic migrants in September, including charadriids known as potential LPAI reservoirs, should create a pulse in influenza occurrences in Afrotropical species.

While exploring these fundamental assumptions for the first time, we also provide a wealth of new and useful information on AIV and wild birds in southern Africa. Our results suggest that none of our starting assumptions can be strongly supported. Some re-thinking of prevailing assumptions about influenza A viruses in southern African bird populations thus appears necessary in planning health care and risk management strategies.

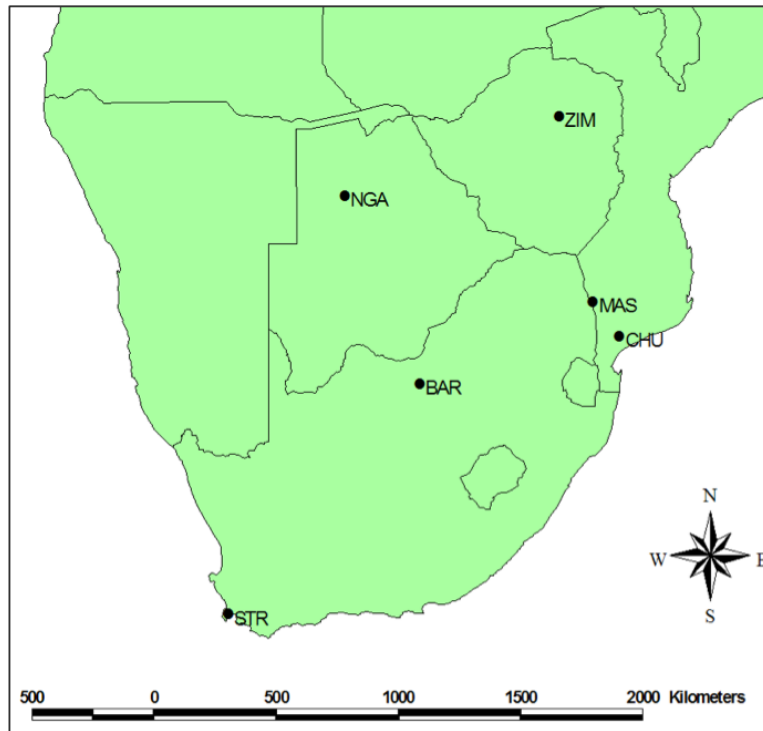
Methods

Project Design and Field Sites

Data were collected in Botswana, Mozambique, South Africa and Zimbabwe from March 2007 to May 2009. We worked at 5 different sites (Figure A5.1) and 12-15 sampling locations per site. We counted and sampled birds at daily, bimonthly, and annual time scales. Our three core sites (Barberspan and Strandfontein in South Africa, and the Manyame catchment in Zimbabwe) were sampled every two months and our Botswana site (Lake Ngami) and Mozambique site (Lake Chuali) every four months. Dates and coordinates of sampling are given in Appendices S1 and S2 (in Supporting Information) and additional information on study sites in Appendix A5.S3.

Figure A5.1: Map of southern Africa showing sampling sites mentioned in this paper. Site codes: ZIM or ZW, Lakes Chivero and Manyame; NGA, Lake Ngami; MAS, Massingir Dam; CHU, Lake Chualali; BAR, Barberspan; STR, Strandfontein. Our three core sites were STR, BAR and ZIM, which fall in different biomes along a north-south latitudinal gradient.

Figure 1



Counting protocols

Each site visit included 5-7 days of standardized bird counts followed by 7-10 days of bird captures in the same locations. Counts consisted of a 10-minute habituation period followed by a 30-minute counting period, during which the number and species of all birds within a 150m radius of the (stationary) observers were recorded. Each location was counted at 4 different times of day over a 5-day period prior to captures (additional details are in Appendix A5.S4). Over the two years of the study we completed 2,503 half-hour point counts. For each of our three core sites (Barberspan, Manyame/Chivero, and Strandfontein) the count data also provide estimates from 13 different points in time (i.e., every two months for two years), giving us a spatiotemporally balanced sampling design for exploring both spatial and temporal variation in the bird community.

Capture and sampling protocols

After the counts we spent 5-10 days catching and sampling birds, using standard procedures as detailed in Appendix A5.S4 (and Figure A5.2). We targeted ducks because they are considered the primary vectors of influenza in Europe and Asia. In addition to ancillary data (morphometry, photographs, blood, feathers) we collected two cloacal and two tracheal swabs per bird. Birds recaptured in the same week were not resampled. All swabs were placed in cryovials in viral transport medium (Hank's salt solution with antibiotics and fungicides) and frozen in liquid nitrogen within half an hour of collection.

The swabs were stored in a -70 freezer and transported in dry ice or liquid nitrogen to an FAO reference laboratory, either ARC-OVI (the Agricultural Research Council-Onderstepoort Veterinary Institute, Pretoria, South Africa) or IZSve (Istituto Zooprofilattico Sperimentale delle Venezie, Padova, Italy) for analysis (see Appendix A5.S5 for details). Sets of swabs were randomised by laboratory; each received the first cloacal and second tracheal

Figure A5.2: Example of a walk-in trap used to catch ducks. In this picture, XX (L) and YY (R) capture Egyptian geese at Strandfontein.

Figure 2



swab from one bird and the second cloacal and first tracheal swab from the next bird. All samples from Botswana and Mozambique were analysed at IZSVe.

Sources of error included (1) failure to obtain a full complement of swabs, due to bird escapes or shortages of vials; (2) labelling errors; (3) loss or destruction of vials in transit; and (4) mistakes in allocation of vials to laboratories. Most of these errors were random and hence unbiased. We had fewer than 4 swabs per bird in just under 4% of cases. Samples were only sent to IZSVe on completion of the project, giving a delay between sampling and analysis of 2-24 months that may have affected the probability of AIV detection (Forster et al. 2008).

Data Analysis

Viral prevalence was too low to determine the influence of the number of swabs on viral detection probability. Since missing swabs were <4% and randomly distributed by species, we assumed that each sampled bird (rather than each swab) had an equal chance of viral detection. Prevalence was calculated as the ratio of the number of influenza viruses detected to the number of birds sampled. Since recaptures were not re-sampled during the same capture mission, and since each sampling effort was at least two months apart, we treated samples from recaptures (including birds that we had ringed and those ringed by others) as independent.

Having quantified viral prevalence for each species by site, we calculated overall prevalence for all bird species and all sites by dividing the total number of birds sampled by the number of samples that were positive for avian influenza viruses. Bird count summaries by site used the average number of birds counted across all point counts.

For the palearctic migrant analysis we included all birds found in our study sites that were both listed in class 6 (i.e., intercontinental and marine migrants) of the Roberts' database (Hockey et al. 2004) and associated with wetland and estuarine habitats. The total number of

foraging and non-foraging palearctic migrants for each sampling mission was converted to a mean abundance and we used Spearman's rank-order correlations to test for a significant relationship between these data, the abundance of anatids, and viral prevalence. Lastly, we multiplied data on prevalence by avian abundance to estimate the relative abundance of infected birds observed during an average half-hour point count.

Results

We sampled a total of 4,977 birds of 165 different species, including 158 recaptures. Captures were distributed unevenly across sites (Table A5.1) despite comparable sampling effort, with the Zimbabwean site yielding the most birds (n=1916), followed by Barberspan (n=1418) and Strandfontein (n=888). A full listing of the number of individuals of the 165 sampled species is provided in Table A5.S1.

Out of 4,977 sampled birds, 125 were positive for the presence of RNA from the influenza A virus group, giving a prevalence across all species and sites of 2.51%. The probability of an influenza-positive sample being from a cloacal or a tracheal swab was almost identical (n=125, $p=0.48$ vs. $p=0.52$ for cloacal and tracheal swabs respectively). Prevalence across different bird families was uneven (Table A5.2), with four families (Anatidae, Jacanidae, Charadriidae and Dendrocygnidae) together contributing 72.8% of positive samples; the same four families represented 67.5% of birds captured (Tables A5.2 and A5.3).

Reliable conclusions cannot be drawn from small sample sizes. We sampled over 20 individuals (i.e., the influence of an outlier was 5% or less) for 18 different bird families. From these families the highest mean prevalence values across all sites occurred in the

Table A5.1: Numbers of birds sampled for avian influenza, by family and by site. BAR, Barberspan; CHU, Chuali; MAS, Massingir; NGA, Ngami; STR, Strandfontein; ZW, Zimbabwe (Chivero and Manyame). Sample sizes for Anatidae were similar across our three core sites (BAR, ZW, STR). Obvious differences include Jacanidae (jacanas; mostly ZW and CHU), Dendrocygnidae (whistling ducks; mostly ZW) and Rallidae (coots and rails; mostly BAR).

Family	BAR	CHU	MAS	NGA	STR	ZW	Total
Accipitridae	0	0	0	0	2	1	3
Alaudidae	6	0	0	1	0	17	24
Alcedinidae	0	3	1	0	0	9	13
Anatidae	696	27	0	69	680	698	2170
Apodidae	1	0	0	0	0	0	1
Ardeidae	6	14	0	3	27	35	85
Burhinidae	1	1	0	0	0	1	3
Caprimulgidae	0	0	1	1	0	3	5
Cerylidae	1	10	0	4	0	25	40
Charadriidae	99	31	10	79	17	225	461
Ciconiidae	0	0	0	1	0	0	1
Cisticolidae	0	0	0	0	0	5	5

Columbidae	4	0	0	48	26	44	122
Coraciidae	0	0	0	0	0	4	4
Dacelonidae	0	0	0	0	0	8	8
Dendrocygnidae	5	13	1	9	0	206	234
Estrildidae	0	0	0	2	0	5	7
Fringillidae	0	0	0	0	0	1	1
Glareolidae	0	13	3	79	0	21	116
Haematopodidae	0	0	0	0	4	0	4
Hirundinidae	0	2	0	1	3	7	13
Indicatoridae	0	0	0	0	0	1	1
Jacanidae	1	116	0	39	0	337	493
Laniidae	1	0	0	0	0	2	3
Laridae	3	0	0	2	42	16	63
Lybiidae	1	0	0	0	0	2	3
Malaconotidae	0	0	0	0	0	2	2
Meropidae	0	0	0	1	0	0	1
Motacillidae	2	1	0	0	10	30	43
Muscicapidae	1	0	0	1	1	1	4
Numididae	10	0	0	0	8	5	23

Passeridae	2	0	0	1	3	2	8
Phalacrocoracidae	0	1	0	0	5	2	8
Phasianidae	2	0	0	8	7	3	20
Phoenicopteridae	7	0	0	0	0	0	7
Ploceidae	25	17	0	32	10	81	165
Podicipedidae	0	1	0	0	0	1	2
Pycnonotidae	2	0	0	0	3	3	8
Rallidae	491	2	0	1	13	7	514
Recurvirostridae	4	0	0	4	8	0	16
Rostratulidae	1	6	0	25	0	0	32
Scolopacidae	36	11	0	48	0	86	181
Sturnidae	0	0	1	4	2	9	16
Sylviidae	3	2	0	1	0	7	13
Threskiornithidae	1	0	0	3	15	1	20
Tytonidae	4	0	0	0	1	2	7
Upupidae	2	0	0	0	0	1	3
Zosteropidae	0	0	0	0	1	0	1
TOTALS	1418	271	17	467	888	1916	4977

Table A5.2: Numbers of birds that tested positive for avian influenza, by family and site. Information on viral strains is given in Table A5.4. BAR, Barberspan; CHU, Chuali; MAS, Massingir; NGA, Ngami; STR, Strandfontein; ZW, Zimbabwe.

Family	BAR	CHU	MAS	NGA	STR	ZW	Total Positives	Total captures	Total prevalence %
Accipitridae	0	0	0	0	0	0	0	3	0
Alaudidae	0	0	0	0	0	3	3	24	12.5
Alcedinidae	0	0	0	0	0	1	1	13	7.7
Anatidae	8	0	0	1	8	35	52	2170	2.4
Apodidae	0	0	0	0	0	0	0	1	0
Ardeidae	0	0	0	0	0	0	0	85	0
Burhinidae	0	0	0	0	0	0	0	3	0
Caprimulgidae	0	0	0	0	0	0	0	5	0
Cerylidae	0	0	0	0	0	1	1	40	2.5
Charadriidae	0	0	0	0	0	12	12	461	2.6
Ciconiidae	0	0	0	0	0	0	0	1	0
Cisticolidae	0	0	0	0	0	0	0	5	0
Columbidae	0	0	0	0	0	0	0	122	0
Coraciidae	0	0	0	0	0	0	0	4	0

Dacelonidae	0	0	0	0	0	0	0	8	0
Dendrocygnidae	0	0	0	2	0	10	12	234	5.1
Estrildidae	0	0	0	0	0	0	0	7	0
Fringillidae	0	0	0	0	0	0	0	1	0
Glareolidae	0	0	0	0	0	0	0	116	0
Haematopodidae	0	0	0	0	0	0	0	4	0
Hirundinidae	0	0	0	0	0	1	1	13	7.7
Indicatoridae	0	0	0	0	0	0	0	1	0
Jacanidae	0	0	0	0	0	15	15	493	3.0
Laniidae	0	0	0	0	0	0	0	3	0
Laridae	0	0	0	0	0	0	0	63	0
Lybiidae	0	0	0	0	0	0	0	3	0
Malaconotidae	0	0	0	0	0	0	0	2	0
Meropidae	0	0	0	0	0	0	0	1	0
Motacillidae	0	0	0	0	0	2	2	43	4.7
Muscicapidae	0	0	0	0	0	0	0	4	0
Numididae	1	0	0	0	0	0	1	23	4.3
Passeridae	1	0	0	0	0	0	1	8	12.5
Phalacrocoracidae	0	0	0	0	0	0	0	8	0
Phasianidae	0	0	0	0	0	0	0	20	0
Phoenicopteridae	0	0	0	0	0	0	0	7	0

Ploceidae	0	0	0	0	0	5	5	165	3.0
Podicipedidae	0	0	0	0	0	0	0	2	0
Pycnonotidae	1	0	0	0	0	0	1	8	12.5
Rallidae	7	0	0	0	0	0	7	514	1.4
Recurvirostridae	0	0	0	0	0	0	0	16	0
Rostratulidae	0	0	0	0	0	0	0	32	0
Scolopacidae	0	0	0	0	0	7	7	181	3.9
Sturnidae	0	0	0	0	0	0	0	16	0
Sylviidae	0	0	0	0	0	2	2	13	15.4
Threskiornithidae	0	0	0	0	1	0	1	20	5
Tytonidae	0	0	0	0	0	0	0	7	0
Upupidae	1	0	0	0	0	0	1	3	33.3
Zosteropidae	0	0	0	0	0	0	0	1	0
TOTALS	19	0	0	3	9	94	125	4977	

Table A5.3: Prevalence of avian influenza by avian family and by site over the period March 2007-April 2009. This table is derived from information given in Table A5.1 and Table A5.2. BAR, Barberspan; CHU, Chuali; MAS, Massingir; NGA, Ngami; STR, Strandfontein; ZW, Zimbabwe.

Family	BAR	CHU	MAS	NGA	STR	ZW	Total	Total
							Captures	Positives
Accipitridae	0.0	0.0	0.0	0.0	0.0	0.0	3	0
Alaudidae	0.0	0.0	0.0	0.0	0.0	17.6	24	3
Alcedinidae	0.0	0.0	0.0	0.0	0.0	11.1	13	1
Anatidae	1.1	0.0	0.0	1.4	1.2	5.0	2170	52
Apodidae	0.0	0.0	0.0	0.0	0.0	0.0	1	0
Ardeidae	0.0	0.0	0.0	0.0	0.0	0.0	85	0
Burhinidae	0.0	0.0	0.0	0.0	0.0	0.0	3	0
Caprimulgidae	0.0	0.0	0.0	0.0	0.0	0.0	5	0
Cerylidae	0.0	0.0	0.0	0.0	0.0	4.0	40	1
Charadriidae	0.0	0.0	0.0	0.0	0.0	5.3	461	12
Ciconiidae	0.0	0.0	0.0	0.0	0.0	0.0	1	0
Cisticolidae	0.0	0.0	0.0	0.0	0.0	0.0	5	0
Columbidae	0.0	0.0	0.0	0.0	0.0	0.0	122	0

Coraciidae	0.0	0.0	0.0	0.0	0.0	0.0	4	0
Dacelonidae	0.0	0.0	0.0	0.0	0.0	0.0	8	0
Dendrocygnidae	0.0	0.0	0.0	22.2	0.0	4.9	234	12
Estrildidae	0.0	0.0	0.0	0.0	0.0	0.0	7	0
Fringillidae	0.0	0.0	0.0	0.0	0.0	0.0	1	0
Glareolidae	0.0	0.0	0.0	0.0	0.0	0.0	116	0
Haematopodidae	0.0	0.0	0.0	0.0	0.0	0.0	4	0
Hirundinidae	0.0	0.0	0.0	0.0	0.0	14.3	13	1
Indicatoridae	0.0	0.0	0.0	0.0	0.0	0.0	1	0
Jacanidae	0.0	0.0	0.0	0.0	0.0	4.5	493	15
Laniidae	0.0	0.0	0.0	0.0	0.0	0.0	3	0
Laridae	0.0	0.0	0.0	0.0	0.0	0.0	63	0
Lybiidae	0.0	0.0	0.0	0.0	0.0	0.0	3	0
Malaconotidae	0.0	0.0	0.0	0.0	0.0	0.0	2	0
Meropidae	0.0	0.0	0.0	0.0	0.0	0.0	1	0
Motacillidae	0.0	0.0	0.0	0.0	0.0	6.7	43	2
Muscicapidae	0.0	0.0	0.0	0.0	0.0	0.0	4	0
Numididae	10.0	0.0	0.0	0.0	0.0	0.0	23	1
Passeridae	50.0	0.0	0.0	0.0	0.0	0.0	8	1

Phalacrocoracidae	0.0	0.0	0.0	0.0	0.0	0.0	8	0
Phasianidae	0.0	0.0	0.0	0.0	0.0	0.0	20	0
Phoenicopteridae	0.0	0.0	0.0	0.0	0.0	0.0	7	0
Ploceidae	0.0	0.0	0.0	0.0	0.0	6.2	165	5
Podicipedidae	0.0	0.0	0.0	0.0	0.0	0.0	2	0
Pycnonotidae	50.0	0.0	0.0	0.0	0.0	0.0	8	1
Rallidae	1.4	0.0	0.0	0.0	0.0	0.0	514	7
Recurvirostridae	0.0	0.0	0.0	0.0	0.0	0.0	16	0
Rostratulidae	0.0	0.0	0.0	0.0	0.0	0.0	32	0
Scolopacidae	0.0	0.0	0.0	0.0	0.0	8.1	181	7
Sturnidae	0.0	0.0	0.0	0.0	0.0	0.0	16	0
Sylviidae	0.0	0.0	0.0	0.0	0.0	28.6	13	2
Threskiornithidae	0.0	0.0	0.0	0.0	6.7	0.0	20	1
Tytonidae	0.0	0.0	0.0	0.0	0.0	0.0	7	0
Upupidae	50.0	0.0	0.0	0.0	0.0	0.0	3	1
Zosteropidae	0.0	0.0	0.0	0.0	0.0	0.0	1	0

Alaudidae (larks; 24 birds, 3 positives, prevalence 12.5%) and the Dendrocygnidae (whistling ducks; 234 birds, 12 positives, prevalence 5.15%). Also of note were the Scolopacidae (sandpipers and snipes, 180 birds, 6 positives, prevalence 3.33%), Jacanidae (jacanas, 492 birds, 15 positives, prevalence=3.05%), Ploceidae (weavers, 165 birds, 5 positives, prevalence=3.03%), Charadriidae (plovers and lapwings; 458 birds, 12 positives, prevalence=2.62%), and Anatidae (ducks; 2168 birds, 52 positives, prevalence=2.4%). Conversely, despite reasonably large sample sizes, no AIV RNA was found in the Columbidae (pigeons and doves; n=122), Glareolidae (pratincoles and coursers; n=116) or Ardeidae (herons, egrets, and bitterns; n=88).

There was no spatial synchrony in influenza occurrences, with the prevalence of LPAI viruses in any two-month sampling period not being significantly correlated between any pair of sites (n=12 or 13, Spearman's $\rho < 0.43$, p not significant to the 0.05 or 0.1 levels in all cases).

Two influenza viruses were isolated and several different strains identified (Table A5.4). An H1N8 influenza virus was isolated from an Egyptian goose *Alopochen aegyptiacus* caught at Barberspan and an H3N8 influenza virus from a Red-billed teal *Anas erythrorhyncha* caught at Strandfontein. Type-related information was obtained via rRT-PCR for an additional 22 viruses, which included 10 H5-positive and 10 H7-positive samples as well as two H6-positives. Amplicons from the reactions were insufficient for obtaining DNA sequences, and thus the amino acid sequence at the HA0 cleavage sites could not be determined; it is therefore unknown whether the H5 and H7 viruses were of high or low pathogenicity. H7 strains were only identified from Zimbabwe but were found in 5 different species.

Table 5A.4: *Information on viral strains and types. This table describes birds that tested positive, rather than positive samples; the 6 birds that tested positive for the same type on two different swabs provide 6 entries rather than 12. Blank cells are zeros rather than unknown values.*

Common name	Latin name	Family	Total +ves	H1 +ve	H3 +ve	H5 +ve	H6 +ve	H7 +ve	Typed
African hoopoe	<i>Upupa africana</i>	Upupidae	1						
African jacana	<i>Actophilornis africanus</i>	Jacanidae	15			2			
African pipit	<i>Anthus cinnamomeus</i>	Motacillidae	1						
African red-eyed bulbul	<i>Pycnonotus nigricans</i>	Pycnonotidae	1			1			
African snipe	<i>Gallinago nigripennis</i>	Scolopacidae	1						
African wattled lapwing	<i>Vanellus senegallus</i>	Charadriidae	3					1	
Barn swallow	<i>Hirundo rustica</i>	Hirundinidae	1						
Blacksmith lapwing	<i>Vanellus armatus</i>	Charadriidae	8			1		1	
Cape teal	<i>Anas capensis</i>	Anatidae	1						

Chestnut-backed sparrowlark	<i>Eremopterix leucotis</i>	Alaudidae	3			1			
Common ringed plover	<i>Charadrius hiaticula</i>	Charadriidae	1						
Common sandpiper	<i>Actitis hypoleucos</i>	Scolopacidae	2						
Egyptian goose	<i>Alopochen aegyptiaca</i>	Anatidae	7	1					H1N8
Fulvous duck	<i>Dendrocygna bicolor</i>	Dendrocygnidae	2				1		
Glossy ibis	<i>Plegadis falcinellus</i>	Threskiornithidae	1						
Helmeted guineafowl	<i>Numida meleagris</i>	Numididae	1						
Hottentot teal	<i>Anas hottentota</i>	Anatidae	3			1			
Little rush- warbler	<i>Bradypterus baboecala</i>	Sylviidae	1						
Little stint	<i>Calidris minuta</i>	Scolopacidae	3					1	
Malachite kingfisher	<i>Alcedo cristata</i>	Alcedinidae	1			1			
Pied kingfisher	<i>Ceryle rudis</i>	Cerylidae	1						

Red-billed quelea	<i>Quelea quelea</i>	Ploceidae	1						
Red-billed teal	<i>Anas erythrorhyncha</i>	Anatidae	35		1			2	H3N8
Red- knobbed coot	<i>Fulica cristata</i>	Rallidae	7						
South African shelduck	<i>Tadorna cana</i>	Anatidae	2			1	1		
Southern grey-headed Sparrow	<i>Passer diffusus</i>	Passeridae	1						
Southern masked- weaver	<i>Ploceus velatus</i>	Ploceidae	1						
Spur- winged goose	<i>Plectropterus gambensis</i>	Anatidae	2						
Village weaver	<i>Ploceus cucullatus</i>	Ploceidae	2						
White-faced duck	<i>Dendrocygna viduata</i>	Dendrocygnidae	10			1		5	
Willow warbler	<i>Phylloscopus trochilus</i>	Sylviidae	1						

Wood sandpiper	<i>Tringa glareola</i>	Scolopacidae	1			1			
Yellow bishop	<i>Euplectes capensis</i>	Ploceidae	1						
Yellow- billed duck	<i>Anas undulata</i>	Anatidae	2						
Yellow- throated longclaw	<i>Macronyx croceus</i>	Motacillidae	1						
Totals			125	1	1	10	2	10	(2)

Table A5.5: Palearctic migrants observed during point counts. The ‘total’ column sums foraging and non-foraging birds (e.g. whimbrel were only observed in flight). Abundance by location were divided by four to average over different samples, but since total numbers of birds are more relevant to the role of palearctic migrants in influenza transmission, we have not divided by the number of locations per site.

Common name	Latin name	Family	Total
Amur falcon	<i>Falco amurensis</i>	Falconidae	27
Barn swallow	<i>Hirundo rustica</i>	Hirundinidae	1847
Black-tailed godwit	<i>Limosa limosa</i>	Scolopacidae	0
Common greenshank	<i>Tringa nebularia</i>	Scolopacidae	89
Common redshank	<i>Tringa totanus</i>	Scolopacidae	0
Common ringed plover	<i>Charadrius hiaticula</i>	Charadriidae	57
Common sandpiper	<i>Actitis hypoleucos</i>	Scolopacidae	138
Common swift	<i>Apus apus</i>	Apodidae	18
Common tern	<i>Sterna hirundo</i>	Laridae	49
Common whimbrel	<i>Numenius phaeopus</i>	Scolopacidae	0
Curlew sandpiper	<i>Calidris ferruginea</i>	Scolopacidae	372
Eurasian curlew	<i>Numenius arquata</i>	Scolopacidae	1
European roller	<i>Coracias garrulus</i>	Coraciidae	4

Green sandpiper	<i>Tringa ochropus</i>	Scolopacidae	11
Grey plover	<i>Pluvialis squatarola</i>	Charadriidae	2
Lesser black-backed gull	<i>Larus fuscus</i>	Laridae	2
Lesser grey shrike	<i>Lanius minor</i>	Laniidae	1
Lesser kestrel	<i>Falco naumanni</i>	Falconidae	2
Little stint	<i>Calidris minuta</i>	Scolopacidae	1741
Marsh sandpiper	<i>Tringa stagnatilis</i>	Scolopacidae	44
Marsh warbler	<i>Acrocephalus palustris</i>	Sylviidae	0
Montagu's harrier	<i>Circus pygargus</i>	Accipitridae	3
Red-backed Shrike	<i>Lanius collurio</i>	Laniidae	4
Ruddy turnstone	<i>Arenaria interpres</i>	Scolopacidae	1
Ruff	<i>Philomachus pugnax</i>	Scolopacidae	1728
Sand martin	<i>Riparia riparia</i>	Hirundinidae	81
Sanderling	<i>Calidris alba</i>	Scolopacidae	15
Sandwich tern	<i>Sterna sandvicensis</i>	Laridae	672
Sedge warbler	<i>Acrocephalus schoenobaenus</i>	Sylviidae	3
Steppe buzzard	<i>Buteo vulpinus</i>	Accipitridae	1
Terek sandpiper	<i>Xenus cinereus</i>	Scolopacidae	2
White-winged tern	<i>Chlidonias leucopterus</i>	Laridae	658

Willow warbler	<i>Phylloscopus trochilus</i>	Sylviidae	5
Wood sandpiper	<i>Tringa glareola</i>	Scolopacidae	458
Yellow wagtail	<i>Motacilla flava</i>	Motacillidae	7

Influenza viruses are in circulation across the subregion throughout the year (Figure A5.3), with no obvious pattern in relation to temperature or rainfall. Patterns between years also appear to be inconsistent, with peaks in viral prevalence in December 2007 and January 2008 in Zimbabwe and Barberspan not present in 2008-2009.

These data should be interpreted within the context of the sampled bird communities. We had relatively high numbers of influenza-positive birds from each of four avian families: Anatidae, Charadriidae, Dendrocygnidae, and Jacanidae. The birds in each of these families show differing seasonal trends in abundance as well as considerable spatial variation between our three core sites (Figure A5.4).

For the Anatidae, we were able to obtain an estimate of changes in the relative abundance of influenza viruses across our three sites at different times of year (Figure A5.5). This figure presents a different portrayal of viral risk from a simple prevalence estimate, as well as giving a rough guide to the number of infected birds that occur in an average point count at each site. Using additional bird count data, such as the African Waterfowl Census, these data could be extrapolated to estimate the total number of birds infected with LPAI in our study sites at different times of year.

During counts we recorded 32,153 individuals belonging to 32 different palearctic migrant bird species from 12 avian families (Table A5.5). Numbers of palearctic migrants showed a strong peak in the southern African summer (Figure A5.6) with varying levels of between-site consistency between years. Comparison of the abundance of palearctic migrants and the prevalence of viruses from the same site and time, treating each site as an independent sample at each time step, found no dependency of viral prevalence on numbers of migrants (Spearman's $r=0.039$, $p<0.8$, $n=42$). Numbers of anatid ducks were also independent of viral prevalence (Spearman's $r=-0.1$, $p<0.5$, $n=42$). At time lags of 2 and 4 months, and excluding

the Lake Ngami data, the relationship remained insignificant (2 months, Spearman's $r=0.2$, $p<0.22$, $n=35$; and at 4 months, $r=0.1$, $p<0.57$, $n=33$).

Discussion

The overall prevalence of LPAI influenza viruses that we found in anatid ducks across southern Africa is 2.4%. The range in PCR prevalence in anatids reported from Northern Europe is between 2.1% and 3.8% (Munster et al. 2006, Munster and Fouchier 2009); and an extended survey in EU member states documented an overall LPAI prevalence in Europe of 1.87% (Breed et al. 2007). Some studies have found higher prevalences, ranging from 4% in Switzerland (Baumer et al. 2010) through 6.1% for European dabbling ducks (Munster et al. 2007) to as high as 12-15% (Olsen et al. 2006, Terregino et al. 2007, Wallensten et al. 2007). Estimates depend on the time of year when sampling occurred and the species that were tested (Olsen et al. 2006); our results are within the range of northern hemisphere estimates rather than notably lower.

One of our most interesting results is the lack of a predictable annual spike in prevalence. In Canada, for example, AIV prevalence in anatids may be as high as 60% on breeding grounds in early fall (Olsen et al. 2006). Our highest prevalence across all birds for any one sampling event was 21.43%, in summer in Zimbabwe; but in the same month in the following year, albeit with a relatively small sample size, prevalence was zero (Figure A5.3). We attribute this unpredictability to the relatively stochastic nature of southern African seasonality and the flexible movement strategies of nomadic southern African ducks.

Figure A5.3: Prevalence by site and month across all captured birds. Sites are BAR, Barberspan; STR, Strandfontein; NGA, Ngami; and ZW, Zimbabwe (Manyame and Chivero). Note that (1) another 294 birds were sampled in Mozambique over the same period, with no AIV positives found; and (2) BAR, STR and ZW were sampled every 2 months and NGA every 4 months, so birds were not sampled in some months. The coloured squares at the top of the chart indicate when a given site was sampled, using the same colour codes as the bars.

Figure 3

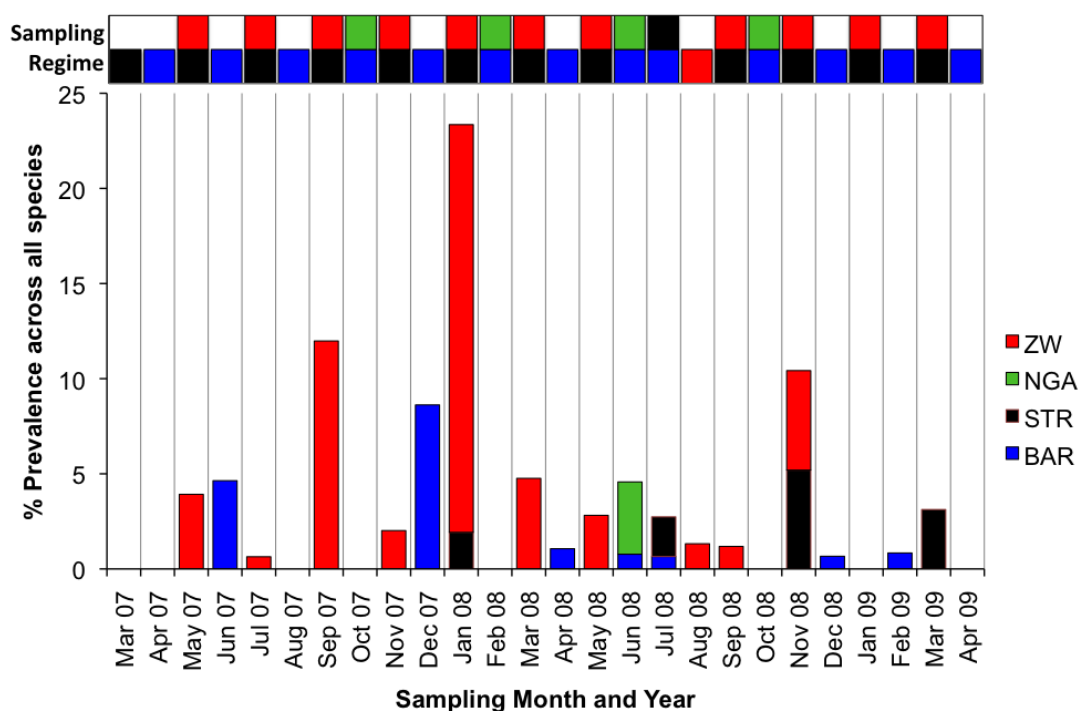


Figure A5.4: Relative abundance of (a) *Anatidae*, (b) *Charadriidae*, (c) *Jacaniidae* and (d) *Dendrocygnidae* at each of the four sites from which we obtained AIV positive samples (Strandfontein, Barberspan, Lakes Manyame and Chivero, and Lake Ngami) as estimated by monthly point counts at each site during the sampling period. We undertook bird counts but not captures at Lake Ngami in February 2009, so that count is not reflected in Figure A5.3.

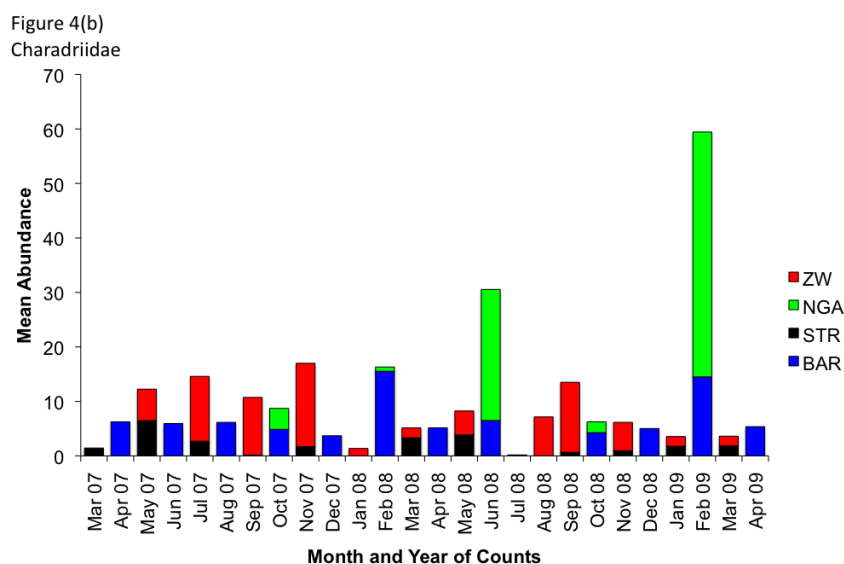
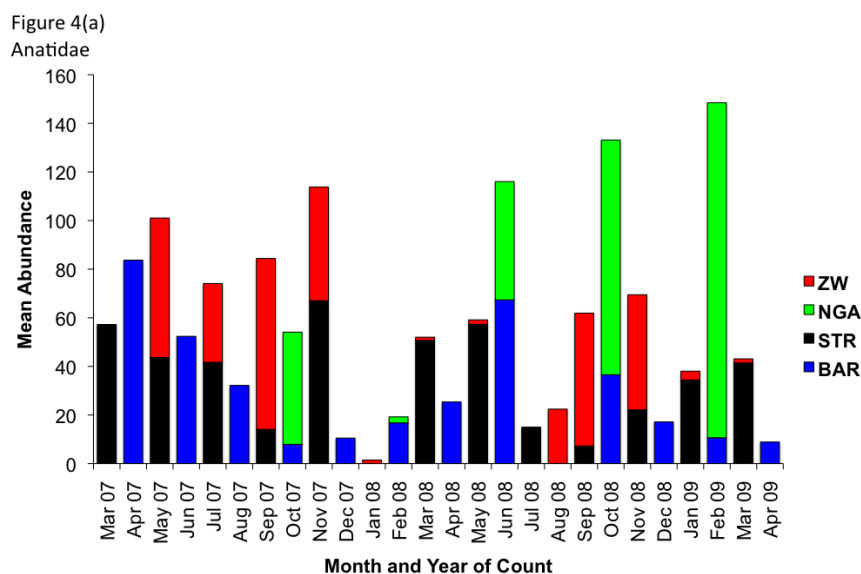


Figure 4(c)
Jacanidae

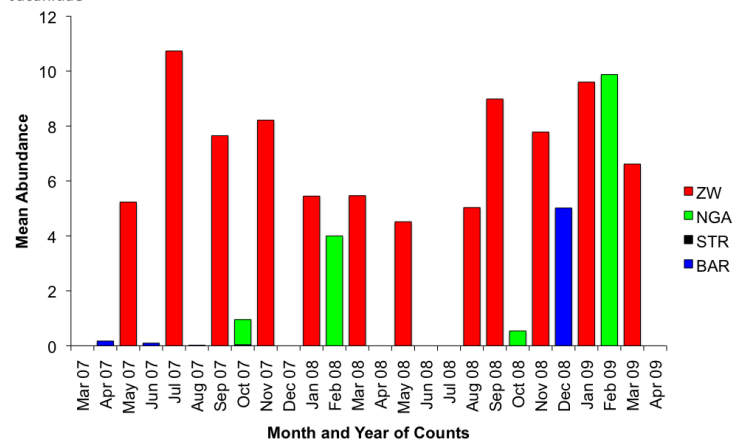


Figure 4(d)
Dendrocygnidae

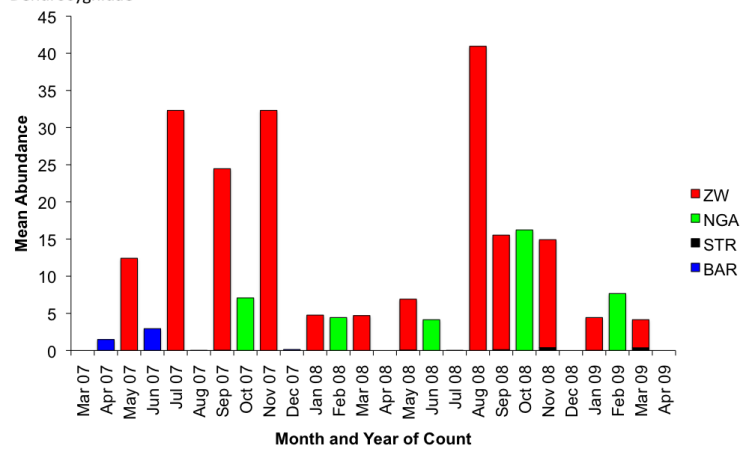


Figure A5.5: Relative abundance of infected anatids per half-hour point count by site and month.

Note how the impression given by this figure differs from that in A5.s 2 and A5.3(a).

Figure 5

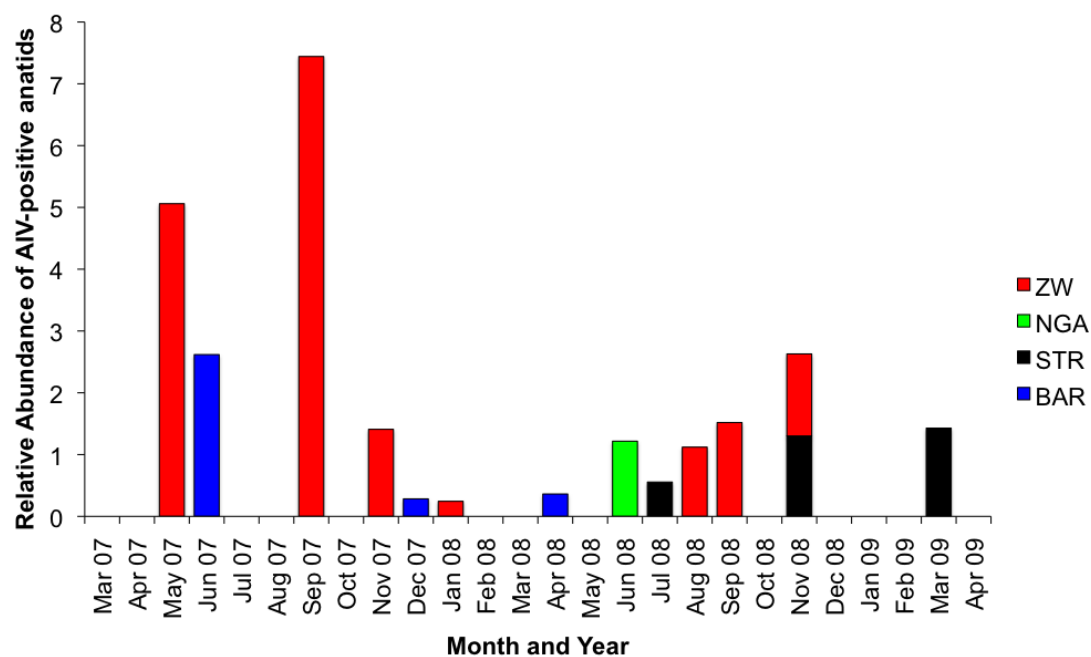
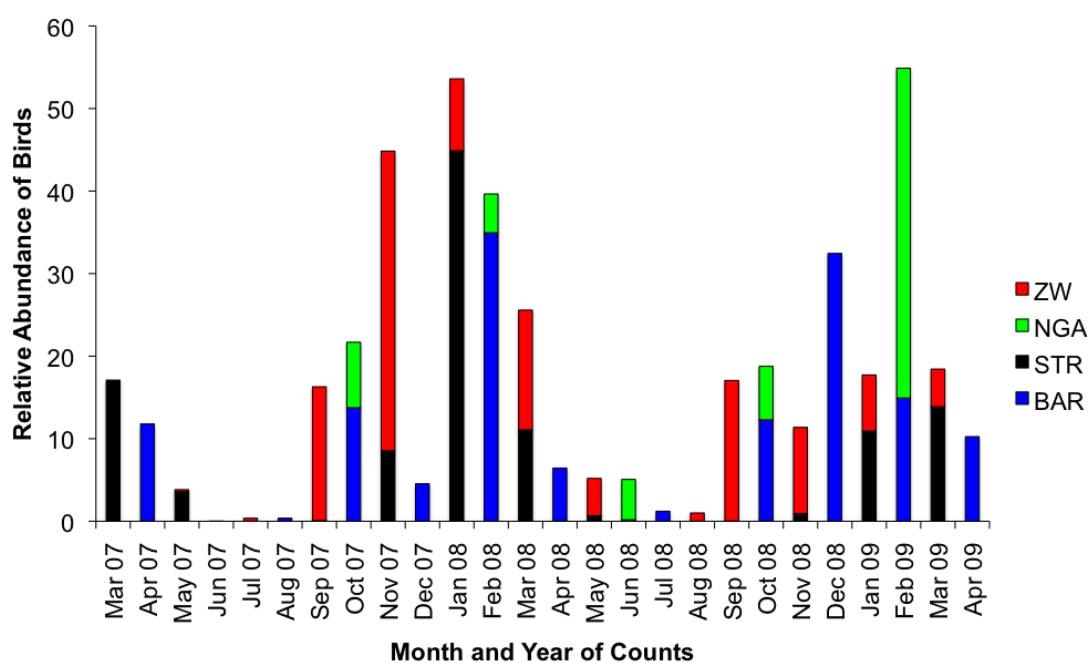


Figure A5.6: *Relative abundance of palearctic migrants per half-hour point count by site. A breakdown by species is given in Table A5.5. Birds arrive earlier at more northern sites (ZW and NGA); seasonal peaks coincide with the austral summer and the boreal winter.*

Figure 6



The prevalence of influenza A viruses in southern Africa appears to be twice as high in dendrocygnid (whistling) ducks (5.2%) as in anatid ducks (2.4%), although this result may be partly an artefact of whistling ducks having been sampled in largest numbers at the site with the highest overall viral prevalence. Most of our dendrocygnid samples were from white-faced duck *Dendrocygna viduata*, but fulvous duck *Dendrocygna bicolor* are common (if almost uncatchable) at Lake Chualali and Lake Ngami. Whistling ducks are less abundant in South Africa but we observed both fulvous and white-backed duck *Thalassornis leuconotus* as far south as Strandfontein, and Barberspan periodically hosts flocks of >20 white-faced duck. White-faced and fulvous duck have an extensive pan-African range and individuals from populations north of the equator may migrate annually to western Europe. Gaidet et al. (2007)(Appendix Two) reported an AI prevalence of 3% in west African dendrocygnids, and Gaidet et al. (2008) found HPAI H5 genomes in white-faced duck in west Africa. Given their high abundance and mobility (Cumming et al. 2008), whistling ducks may play an important regional role in the dynamics of AIV.

Although no HPAI viruses were positively identified, potentially virulent H5 and H7 strains are in circulation in southern Africa in resident wild bird populations. There is some hint of a latitudinal gradient in prevalence, with Manyame > Barberspan > Strandfontein; but data from Mozambique and Botswana do not fit this pattern, although the sample sizes (n=271 and 467 birds respectively) are too small to draw strong inferences.

Most studies of avian influenza have focused on Anseriformes and Charadriiformes (ducks and waders), but other waterbirds may play a role in maintaining AIV in southern Africa. Rallids and jacanids (e.g. Red-knobbed Coot *Actophilornis africanus* and African Jacana *Fulica cristata*) occur year-round in high abundances in many wetlands and frequently forage near to dabbling and diving ducks. Cormorants and darters (Phalacrocoridae) are common in our study sites, mobile, and frequently roost with ducks. Risks of transmission to

humans are increased by their capture in fishing nets. A variety of other species, such as Sacred ibises (*Threskiornis aethiopicus*; Threskiornithidae) also share foraging habitats with grazing and dabbling ducks; Sacred ibises in particular may feed on carcasses, making them potentially vulnerable to AIV epidemics.

For the Passeriformes, a prevalence of 4.5% (14 positives out of 308 birds) suggests a potential role in influenza epidemiology. Most of the AIV positive species that we found in this order are residents (yellow throated longclaw, chestnut-backed sparrowlark, red-billed quelea, and village weaver; Table A5.4) but barn swallows and willow warblers are palearctic migrants. Our data and those from other studies (Chapter Three - Caron et al. 2010) suggest that some passeriform families (e.g. Alaudidae and Ploceidae) may contribute to the persistence and spread of AIV in southern African ecosystems.

In practical terms, our results preclude the assumptions of an annual cycle of viral circulation and strong seasonal variation in wild bird-related risks that hold in many northern hemisphere regions. From a health care perspective, AIV epidemics in wild birds appear to be possible at any time of the year. The opportunistic behavioural responses of waterbird populations to environmental drivers, and the lag between rainfall and bird and pathogen responses may nonetheless make it possible to obtain short-term predictions of AIV risks using information on rainfall.

Supporting Information (not displayed in the thesis)

Additional Supporting Information may be found in the online version of this article:

Appendix A5.S1.: Dates of each sampling mission.

Appendix A5.S2.: Coordinates of all counting and capture sites.

Appendix A5.S3.: Background information on study sites.

Appendix A5.S4.: Additional information on the bird counting and capture protocols.

Appendix A5.S5.: Detailed information on laboratory methods.

Table A5.S1.: The number of individuals of each of the 165 species that we sampled during this study (includes scientific, common, and family names).



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Appendix Six: Epidemiological interaction at the wildlife/livestock/human interface: can we anticipate emerging infectious diseases in their hotspots?

(Appendix reference: Caron, A., de Garine-Wichatitsky, M., Morand. 2012. Chapter 14: Epidemiological interaction at the wildlife/livestock/human interface: can we anticipate emerging infectious diseases in their hotspots? A framework for understanding emerging diseases processes in their hotspots. “New Frontiers of Molecular Epidemiology of Infectious Diseases”. Morand, S., Beaudreau F., Cabaret J. (Eds), Part 5, 311-332.)



Introduction

The incidence of Emerging Infectious Diseases (EIDs) in human and domestic species has increased in the last decades (Cleaveland et al. 2001, Taylor et al. 2001, Jones et al. 2008, Woolhouse 2008). Zoonoses constitute 60.3% of human EIDs of which 71.8% originate in a wildlife source with intermediate animal hosts often necessary (Jones et al. 2008). Following (Haydon et al. 2002), we define target species as those species receiving the bulk of sanitary surveillance: human and domestic species as well as a few wildlife flagship species. The sum of these target species represents a tiny fraction of the biodiversity. The bias induced by this focused surveillance hides the majority of EIDs events in the non-target species.

We have little insight about what is happening “in the wild”, where statistically most of pathogen evolution and transmission processes are underway. Our knowledge about EIDs processes is therefore limited and skewed in favour of a non-random sample of those occurring. Describing these processes and their common properties could benefit the surveillance and control of EID in target but also in non-target species, supporting global objectives of public and animal health and conservation as presented in the “One World, One Health” concept (Karesh and Deem 2000, Osofsky et al. 2008, Gibbs and Anderson 2009).

The origin of this increase in EIDs is complex. A relation with disturbed ecological processes is often assumed (Daszak et al. 2001). Massive changes in organism distribution and relations induced by global trends, such as increased anthropogenic footprint and climate change have triggered new host, pathogen and environment interactions. These new ecological interactions are not randomly geographically located and regions with a higher risk of emergence can be identified by plotting known emergence events on a map. According to Jones et al. 2008, hotspots of disease emergence are characterised by: 1) high densities in human and animal populations in systems under intensive health surveillance; 2) the

wildlife/livestock/human interface in tropical ecosystems. Focusing on wildlife-linked EIDs, the integration of these two characteristics leads to a description of EIDs events as a two steps process: 1) emergence of the pathogen *sensu stricto* (defined as the interspecies spill-over from a non-target to a target species) (steps 1 and 2 in Childs et al. 2007); 2) amplification of the epidemic phase with higher host availability (provided by higher densities). Recent EIDs can be classified accordingly: Ebola in humans, originating from a potential bat reservoir has reached step 1 but not step 2 (Leroy et al. 2005); HPAI H5N1 in poultry with a wild bird origin has reached step 1 and 2 (Webster and Govorkova 2006); SARS in humans, originating in bats, as reached step 1 but had a limited step 2 in 2003 (Wang and Eaton 2007). In tropical and sub-tropical ecosystems, the degree of wildlife/livestock/human interface and biodiversity are high and offer numerous potential events of spill-over. However, low animal and human health surveillance decrease the detection probability. In ecosystems with artificially high host densities, less inter-species transmission events occur because of the physical and sanitary protection of these systems. Moreover, efficient surveillance systems in target species increase the detection probability of the emergence. This analysis could explain the global EID patterns observed.

In order to improve the efficiency and reduce the cost of health interventions, research should be implemented on step 1 in order to understand, predict and control emergence processes and to prevent amplification events (step 2) or prepare the health sector to control them (Barclay 2008). Step 1 requires focusing on the wildlife/livestock/human interface, which necessitates ecological, epidemiological as well as socio-economical information. This interface is characterised by: a) multi-host systems; b) managed and unmanaged animal populations (“managed” referring to the state of animal population when influenced by voluntary human intervention); c) natural and modified habitats; d) remote areas, where basic commodities are often lacking; e) unknown socio-economical system in marginalised human

communities. The lack of baseline information which characterised these interfaces combined with the above particularities requires the development of theoretical and technological tools adapted to the study of EIDs in their hotspots (Morgan et al. 2006). However, studying EIDs in selected ecosystems raises any of the following challenges: a) looking for a still unknown pathogen; b) arriving after the emergence process, when one does not know if the spill-over process still occurs; c) looking for a pathogen which is not yet in the study site. In order to overcome this dilemma, a shift from a pathogen-centred to a process-centred approach is necessary. The process at stake is the pathogen spill-over in multi-hosts system at the wildlife/livestock/human interface.

We define epidemiological interaction (EI) as any ecological interaction resulting in the transmission of pathogen between two host populations (Chapter Three - Caron et al. 2010). This definition of EI is developed in the next sections of this chapter. We then present a research framework, process-orientated, using host and/or pathogen data for the inference of emergence risk in a given ecosystem. We describe a method to build EI network and present using an example how this network can be used to identify host populations or EI at risk for pathogen emergence.

Methods

Estimating transmission rate for pathogens shared between host populations

Standard approaches to study pathogen transmission rate in a single host population are pathogen-centred (Bordes and Morand 2009). In one host population, prevalence and incidence of the pathogen measured by serological or viral detection techniques can be used to calculate the transmission rate. Common index of this transmission rate are the R_0 and β

index and the force of infection (McCallum et al. 2001; see Real and Biek 2007 for a recent discussion of these parameters for wildlife zoonosis). These parameters are defined for a given pathogen in a defined host population. The data needed to estimate these parameters are the contact rate between hosts, the transmission probability resulting from these contacts and the infectiveness of the pathogen in the target host or the probability that such a contact occurs between an infected and a susceptible host (McCallum et al. 2001). Models of a shared pathogen between two hosts populations have been developed (see Tompkins et al. 2002 for some examples).

In an experimental situation using a domestic species, these parameters are accessible. *A posteriori*, after an outbreak, these parameters can be estimated if the relevant data has been timely collected. This data concerns repeated data collection on a pre-determined sample of the animal population and the setting-up of the appropriate environment in which the samples will be adequately stored until laboratory testing. Applied to the wildlife/livestock interface, estimating these parameters is difficult in free-ranging species (Morgan et al. 2006). Capture of wild species is often expensive, implemented in extreme conditions not suitable for sample conservation and unrealistic when the activity needs to be repeated in time on the same animals. Real & Biek (Real and Biek 2007) suggest a possible framework using telemetry (radio and satellite) on wild species combined with sampling survey on wild and domestic species. However they identify limits such as the underestimation of the quantity of contacts if the entire wild population is not marked. Richomme et al. 2006 estimated by direct observations the contact rate and exposure between a domestic ungulate and a wild mountain ungulate and discuss additional limits of such data for the inference of EIs because of the nocturnal behaviour of wild species which cannot be apprehended. The necessity to include in the study the respective sensibility of each species to the pathogen requires also an extrapolation from available data on closely-related species. The role of modelling has been

and will be crucial in the estimation of the behaviour of transmission parameters and their interpretation (Lloyd-Smith et al. 2009). A model allows playing with variables to estimate outcomes impossible to observe in the field. They use the available data to test hypothesis which would be too complex or too costly to test in experimental conditions. Multi-host models have been developed under the form of meta-population pathogen diffusion process in the particular case of multi-host meta-population (Arino et al. 2005). Recently, transmission models of multi-strains with differential transmission pathways (with emphasis on the role of the environment) paved the way for multi-pathogen models (Roche and Rohani 2010). However, the integration of multi-host and multi-pathogen models has yet to be done. This step will be necessary in order to encompass the full complexity of transmission ecology at the wildlife/domestic interface.

The application of these different methods to the case of an unknown pathogen before its emergence in the ecosystem raises new issues. Here, the objective is to identify an unknown emerging pathogen to reach the target population. The emergence event *sensu stricto* that we are trying to detect is the spill-over of an unknown pathogen from an unknown source population to a known naive population. Which non-target host should be studied? For which pathogen should we test it? In this context where no target pathogen is identified, a pathogen-centred approach cannot be implemented.

EI network and selection of host and pathogen community to predict pathogen emergence

The human species, a domestic species (e.g. livestock) or a flagship wild species (e.g. mountain gorilla) can be the target species under study. The unknown pathogen emerging in a given ecosystem will be transmitted to the target species by an unknown non-target species, through direct and/or indirect contacts (Figure A6.1). The main assumption of the research

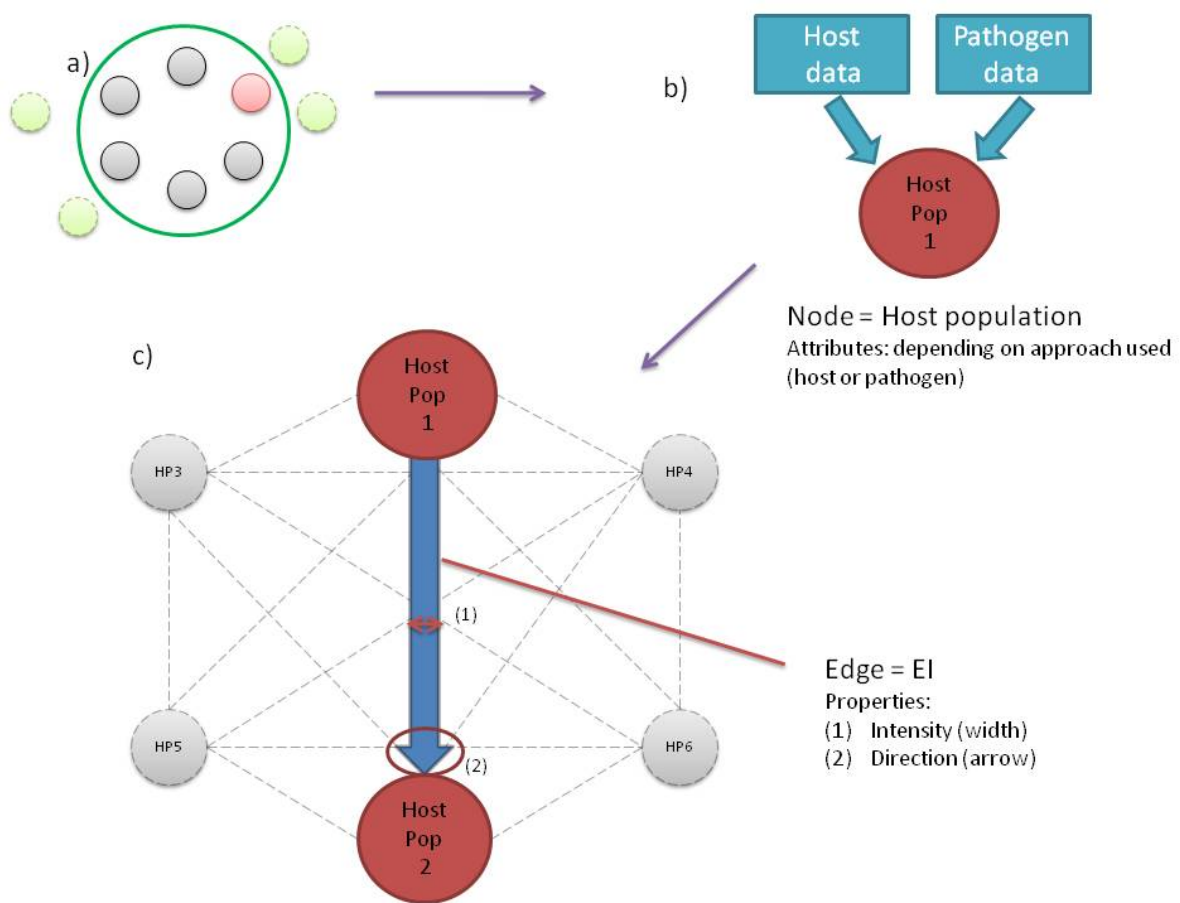
framework is that the emerging pathogen will use contacts between hosts which have already been used by other pathogens. The higher the intensity of EI, the higher the probability of the emerging pathogen to use it. If the EI network can be built between different host populations, prediction on the likelihood of emergence through a particular EI can be made.

Network Analysis (NA) developed primarily in the context of social sciences is increasingly used in health ecology (Luke and Harris 2007). It has been applied to explore relations between cattle movement data (Heath et al. 2008) or human populations patterns (Bansal et al. 2007) and disease spread. A network is a set of different entities defined by “nodes” linked by “edges”. Nodes and edges have several properties (Figure A6.2). Nodes are host populations, and edges represent EIs. Nodes can be linked with characteristics of the host population (ecological or epidemiological) defined by the type of data used to build the network. Edges have two properties: 1) intensity, defined by the number of contacts between host populations, or the proportion of pathogens shared between the two host populations; this property can be graphically represented by the width of the link between 2 nodes; 2) directionality, uni- or bidirectional depending on the transmission possibilities between the two host populations; an arrow at the extremity of the edge represents this property. If interested in the temporal variation of EIs, different networks can be built or different colour for different temporal windows can be used, based on temporal series. Once nodes and edges have been characterised given their attributes, the network illustrates the principal edges-EIs between the target species and non-target species.

Figure A6.1: a) Theoretical contact network including wild, domestic and human populations; in the remaining figures, the same network when the human population (b), a domestic population (c) or a flagship wildlife species (d) is considered as the target species. Depending on the target population selected, the contact network potentially leading to the spill-over of a pathogen from non-target populations change. For example, wildlife population 2 does not play a role in (b) and (c) whereas it is considered as a potential source of pathogen in case (d). Relevant epidemiological interactions in the system change in case (b), (c) and (d).



Figure A6.2: Three-step process to build a EI network and its schematic representation: a) Six Host populations (HP; target – in red- and non-target) are selected in the ecosystem; b) Characterisation of the attributes of the nodes of each HP based on host or pathogen data; c) Zoom on 2 nodes which properties can be decided according to available information (see Box A6.1) and one edge characterised by its 2 properties: (1) Intensity represented by the width of the edge; (2) Direction represented by an arrow which could uni- or bidirectional.



The EI network can be build using two different sources of data: host data or pathogen data. The host data consists in estimates of direct and indirect contacts between host populations. The pathogen data consists in the community of pathogens shared by different host populations. In the former case, the host contacts will be used as estimation of future pathogen spill-over and this approach can be called *a priori*. In the later case, pathogen transmissions that have already occurred will be used to estimate the potential pathways for future pathogen spill-over and the approach is in that case *a posteriori*.

The first step in the building of the EI network is to select which host and which pathogens are included in the study. The non-target host populations represent a range of wild and domestic species living in the same environment as the target species, and potentially acting as candidate source of pathogen. As it is usually impossible to obtain relevant data for all wild species in an area for practical and financial reasons, the selection of potential hosts can be prioritised, using available information: 1) Phylogenetic proximity (e.g. apes most closely related to human or wild bovids species most related to livestock species) is a known criteria to increase the chance of pathogen spill-over between two host species (Davies and Pedersen 2008); 2) Ecological knowledge on host species present in the study site; 3) Epidemiological information can orientate as well the selection process,. For example, bats species represent 25% of all mammal species despite being under-studied from an ecological point of view. They can be in contact for behavioural reasons – feeding, roosting - with different host species. They have also been involved in recent human EIDs epidemiology, notably in haemorrhagic fever events (Wang and Eaton 2007). If the target species is the human species, including bats in the study seems relevant if they occur in the ecosystem. Usually, as most studies focus on a specific or a group of pathogens, the selection of host populations is based on the available knowledge on the pathogen's host range. Caron et al. (submitted) have presented a framework for this selection process based on epidemiological

functional groups of hosts. This framework offers a systematic step by step process to consider and select all potential hosts in a given ecosystem. In this selection process, available data about host contact (e.g. questionnaire-based) or pathogen prevalence (available through national studies) should be used to fine-tune the choice of host populations for the network.

Estimating epidemiological interactions using host data (a priori approach)

Estimates of contacts between the target population and one or more non-target populations can be used to build the EI network. Host movements and contacts can be monitored using different field techniques adapted to the level of study: individual, population or community.

Few empirical studies have considered contacts at the wildlife/livestock interface. At the individual host level, direct observation or telemetry can detect and quantify these contacts. For example, the development of satellite telemetry and the increase capacity of miniature batteries allow the study of animal movements with a large range of body sizes: up to a few grams for birds (Gaidet et al. 2008) at a very fine time-scale (up to a point every 5mn) for long periods (a year or more). Recent examples combining telemetry and density or visual observation techniques have successfully determined contact rate of individuals at the wildlife/livestock interface (Bohm et al. 2009, Perkins et al. 2009). The main weakness of these studies is the underestimation of contacts as it can never be assumed that the entire non-target population has been followed. This weakness can be partially controlled by objective or subjective knowledge of the study site but can never be completely addressed.

At the population or community level, estimates of overlaps in habitat used by different host populations or species (Ezenwa 2003, Kilpatrick et al. 2009) can be utilised as a proxy of interspecies contacts at different seasons. Data collected during road-counts or

counts focusing on key resources or habitat (such as waterholes) on both sides of the interface, as well as trapping data (Caley and Hone 2004) provide a quantitative estimate of interspecies interactions which can be used to build an EI network. . A recent study attempted to estimate EIs in avian communities using bird count data in wild and domestic sites to predict the risk of Avian Influenza transmission at the wildlife/domestic interface (Chapter Three - Caron et al. 2010). Finally, local community questionnaire-based studies can also produce contact rate between host populations, paying particular attention in the design of the questionnaire and treating the information as perceptions and not facts (Brook et al. 2009).

The field of molecular epidemiology has experienced recent and major developments (Gupta et al. 2009). The amount of genetic data on host and pathogen species is produced at an exponential rate (Holmes 2007). Recent years have seen the development of powerful molecular tools to characterize specific pathogens and hosts. This host information supports the characterisation of the genetic distance between and among host species or populations (Nieberding and Olivieri 2007). Host population dynamics estimated by gene flow will be a particular case of population-level study to estimate contacts (see in this book Chevillon et al. 2011, Charbonnel et al. 2011). Molecular tools in this field have been developed and can give indication as fine as the parental relationship between individuals. This genetic data can provide fine information to build an EI network between populations of the same species, giving information on the intensity (or width) of edges as well as the direction of edges.

Most of host contact studies to estimate probabilities of pathogen transmission have been developed in the context of a specific pathogen. Our objective is to use the same tools to qualify and/or quantify EI with no *a priori* on the pathogen under study. As presented in Figure A6.2, after the first step of host selection, each node, representing a host population or species will be attributed characteristics: species and/or population information; and the number of host of the network in contact with the focal species/population (through habitat,

resource overlap or direct contact). Edge intensity will be proportional to the estimation of the contact between the two hosts. Edge direction is usually not indicated except in particular circumstances. For example, species A only transiting in a particular habitat will not be infected in this habitat while species B feeding in this habitat could be infected through environmental contamination. Finally, successive EI networks can be built in order to account for temporal variability of contacts (e.g. seasons). The EI network will have to be interpreted with the same limits as the initial contact data: e.g. contacts between populations will be under-estimated.

Estimating epidemiological interactions using the pathogen level (a posteriori approach)

In the previous paragraph, we presented how host movement and contact data can be used to build an EI network. In the following paragraph, we will show how epidemiological data can also be used to build EI networks using an *a posteriori*, allowing the most likely pathways for the future emergence of new pathogens.

For each pathogen infecting more than one host population, we can estimate EIs; accumulating data on an array of different pathogens will strengthen the network. In other words, gathering epidemiological data on numerous pathogens in an ecosystem in different wild and domestic populations can structure the EI network between these populations and predict to a certain extent (discussed below) the behaviour of an outsider pathogen. The type of data referred to here as already been collected in various studies, albeit not used for this purpose. Jolles et al. 2008 collected data on micro- and macroparasite species in buffaloes in South Africa to investigate potential ecological interactions (competition, synergies) between pathogens in a single host. In another study, Ezenwa 2003 looked at macroparasite richness in sympatric wild and domestic ungulates, and analysed their variations according to habitat

overlap between host species. Other studies dealing with the health status of some endangered species, a parameter increasingly investigated in species conservation, can also produce relevant information on free-ranging species (Philippa et al. 2008).

Parasite community ecology is a subset of community ecology focusing on the distribution of parasites between host populations. Large datasets of parasites (mostly macroparasite) in multi-hosts systems have been collected in the course of these studies (see (Poulin 2007 for a review). Several indices have been used to compare parasite communities between host populations (Poulin 2003, 2010). The Jaccard index (Jaccard 1912) uses presence-absence data of parasites in different host populations to give an indicator of the similarity of these communities. Other indices, more sophisticated, use prevalence data to compare parasite community (Boyle et al. 1990, Poulin 2007). In the context of host populations from the same ecosystem, the geographic distance between the host populations is controlled (geographic distances usually looked at in parasite community ecology are of the order of hundreds or thousands of kilometres). If the phylogenetic distance between host populations can be controlled, the similarities measured can estimate the EI between these host populations. These indices can therefore be used to build EI network as the value of the index between two parasite communities shared by two host populations (their similarity). Analytical tools to control for phylogenetic distances between hosts are already used and new ones are under development incorporating sophisticated statistical analyses (Adams 2008; Hadfield and Nakagawa, 2010).

History of contacts between host populations is another variable to be considered (see discussion). The type of the epidemiological data that one can gather varies depending on the pathogens targeted and the investment of the scientific and private community into the development of specialised diagnostic tools. Presence-absence data are the simplest data that one can gather on pathogen epidemiology in a singular host, and is often the only available

information from the literature. Prevalence data (direct through pathogen detection or indirect through antibody detection) provides the percentage of hosts in each population which have been or are in contact with the pathogens. As mention above, this type of data can be included in the calculation of similarity indices borrowed from community ecology (Boyle et al. 1990, Poulin 2007).

As presented in the previous section, the molecular epidemiology revolution has changed the field of parasite ecology and new genetic information is providing the ground for major advances in pathogen research, evolutionary ecology and population dynamics of pathogens. The characterisation of HIV strains from different human and great apes populations or even from different human individuals has brought important development on our knowledge about the origin and the spread of this pathogen across the world (Heeney et al. 2006, Cohen 2007, Gilbert et al. 2007). Another example is the abundant recent literature on phylogenetic analyses of HPAI H5N1 strains across the globe linking animal and human outbreaks (see Wang et al. 2008, Cattoli et al. 2009 for HPAI H5N1 and Nelson et al. 2007, Liu et al. 2009 for AIV). Other important pathogens have benefited from these technological advances (for some examples on multi-host utilisation (Bastos et al. 2003, Vosloo et al. 2006, Foster et al. 2009). The level of accuracy to detect single nucleotide change in parasite genomes of these molecular tools is increasing and their use at the ecosystem level can pick-up recent transmission events including their direction. Biek et al. 2006 have used the Feline Immunodeficiency virus (FIV) to track the population dynamics of one of its host (*Puma concolor*). Haagmans et al. 2009 highlight this point with another angle and suggest to compare the outcomes of an EIDs outbreak with “closely related pathogens in different but related host species (...)”. The evolution of parasites is often faster than the evolution of their hosts (Nieberding and Olivieri 2007). Recent host population dynamics not detected in the host genetic material can be captured by the faster genetic evolution of its parasites. Applied

to our context, a phylogenetic tree of the same parasites detected in different host populations in the same ecosystem can reveal connexions between these host populations and give an estimate of the EIs between these host populations for this specific pathogen. The molecular tools can be used to characterise EIs. It is likely that in the coming years additional data on genetic of parasites and new analytical methods will provide more power in the estimation of EIs between host populations. Transmission pathways between host populations and EI network will be strengthened by these advances (Chevillon et al. 2011).

Replicating the multi-step process developed in Figure A6.2 and in the previous section, once the host selection has been implemented in the ecosystem, each node representing a host population can be characterised by the total number of parasite species harboured (nodes property represented by its size) and other attributes (e.g. in Box A6.1, the colour of the node is used to indicate in which habitat the host species is mainly occurring). To estimate the intensity of the edge (its width), we use the value of the index calculating the proportion of the parasites shared between two host populations/species. In very rare occasions where knowledge about the epidemiology of some parasites exists, the direction of the EI could be known: e.g. when a species known to be susceptible but not capable of maintaining a parasite is infected by a known reservoir of the parasite. Molecular data can potentially inform EIs on both their properties: intensity, as molecular data can be used as prevalence data (e.g. prevalence for different strains of parasites); direction, the evolution of a parasite strain between two host populations can be tracked back and the donor population can be distinguished from the receptor population. Finally, if time series data is available different network can estimate the variation of EIs across seasons (time series used to infer transmission dynamics between hosts see Begon et al. 1999).

So far, the use of these epidemiological and molecular tools has been limited to one target pathogen and only a few articles have referred to the extension of their use to several

pathogens, to host population dynamics, or to contacts between hosts (Poss et al. 2002). At an ecosystem level, it is the combined use of presence-absence, prevalence and molecular data on multiple pathogens which will define EI networks. The integration of different type of data for different parasite species will be a challenge in the framework of this approach. Prevalence data will give more fine-scale details about the shared community of pathogens than presence/absence data as for each host population, the estimated percentage of individuals in the population infected by the parasite will be taken into account in the index. Molecular data can give even more details about how long ago the parasite strain was transmitted from one host to the other. To our knowledge, the only possibility to integrate this data in one network is to weight each type of data (e.g. giving more weight to molecular, then prevalence and finally presence/absence data). The more data gathered on various pathogens, the more detailed the hypotheses on future EIDs epidemiological pathways.

For example, de Garine-Wichatitsky et al. 2010 (Appendix Three) have recently detected the first case of bovine tuberculosis (bTB) in African buffaloes in a Zimbabwean national park and discussed the outcomes of this emergence at the country level. How will the pathogen behave in this ecosystem? Will it spill-over to the communal cattle populations? To other wildlife species? To the human population? Data on circulating diseases (zoonoses and others) in this area (e.g. foot-and-mouth disease, brucellosis, rift valley fever etc.) can bring information on the structure of the EI network. The network and other epidemiological knowledge about parasites can be used to prioritize the nodes or edges at risk. One can consider interventions on the nodes (host) of the network for surveillance and/or control but also on the edges (EIs) to “break” the transmission pathways and limit the potential for emergence. If not detailed here, the relation between an EI network and a spatially explicit approach can be done.

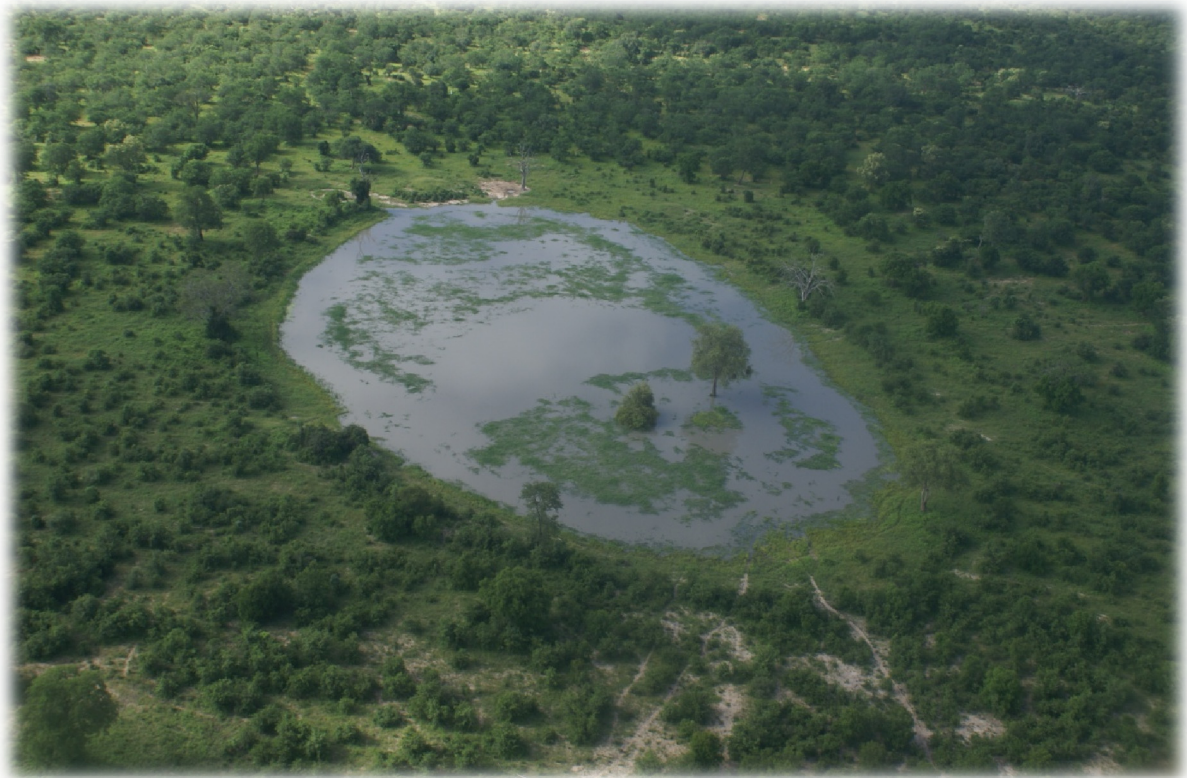
Discussion

The discussion will mainly address the pathogen approach as the host approach is at advance stage of development in the literature.

From an epidemiological perspective, usually investigating one epidemiological cycle at a time, the EI network approach that we present makes no sense. The epidemiology of each disease is different and one cannot draw inferences on a pathogen using data from another. However, from an ecological perspective, the line of thoughts is logical even if challenging. There is not an infinite opportunities for a pathogen to be transmitted between two host populations and new pathogens will use routes already used by other pathogens with a higher probability. Identifying common pathways between host populations will therefore increase the knowledge about future probable emergence pathways in this system. This pathogen approach does not refer to a specific pathogen and focuses on the hosts' direct and indirect contacts but only the ones resulting in pathogen transmission. The utility of such an approach is obvious in the surveillance of EIDs in a hotspot: with an EI network, one can assess the probability of future spill-over processes between specific populations and target surveillance and/or control. In a limited-resource environment (true everywhere but even more in most EID hotspots), EI networks will point at most probable transmission pathways between host populations which can be acted upon to reduce the probability of transmission/emergence.

Box A6.1 (adapted from Chapter Six) gives an example of an EI network built using presence-absence data of macro- and micro- parasites in 14 rodent species and the human species. The data has been gathered from the literature. Using a simple method to describe the parasite communities shared by each pair of host populations, the EI network illustrates the intensity of each edge proportional to its width, the parasite species richness per host and the main habitat where each host occurs. Eco-epidemiological information such as which rodent

host shares a high proportion of its parasite species with humans are visually explicit and new hypotheses about key-rodent species are presented. These observations suggest that the EI network is representative of the level of contacts between the human species and the rodent species. Finally, combining this (crude) epidemiological and ecological information in a single analysis provide more information about the system than a simple juxtaposition of single parasite information.



Box A6.1: *Epidemiological Interaction Network for 14 rodent species and the human species*

In this example, the human species is our target species and we explore the EIs between the human species and several rodent species present in particular ecosystems of Southeast Asia represented by different habitats (dry and irrigated agricultural areas, forests, and villages).

From the literature (Chaisiri et al. 2010, Herbreteau et al. unpublished), we obtained presence-absence data on 14 rodent species. Information about 34 macroparasite species and 8 microparasite species were collected for these 14 rodent species and susceptibility to these parasites for the human species were taken from Chaisiri et al. (2010) and Herbreteau et al. (unpublished) (Table A6.1). A 42 parasites*15 hosts matrix was built (and filled with “1” or “0” for occurrence of infection and absence respectively in each host species. This matrix was used to calculate the Jaccard Index (=number of parasite species present in both host populations/sum of parasite species present in each host populations) displayed in Table A6.2. The Jaccard index value varies therefore between “0” for no parasite species shared and “1” for all parasite species shared.

We used the Jaccard index as a proxy of the epidemiological interactions (EIs) between each host population and built the corresponding EI network (Figure A6.3).

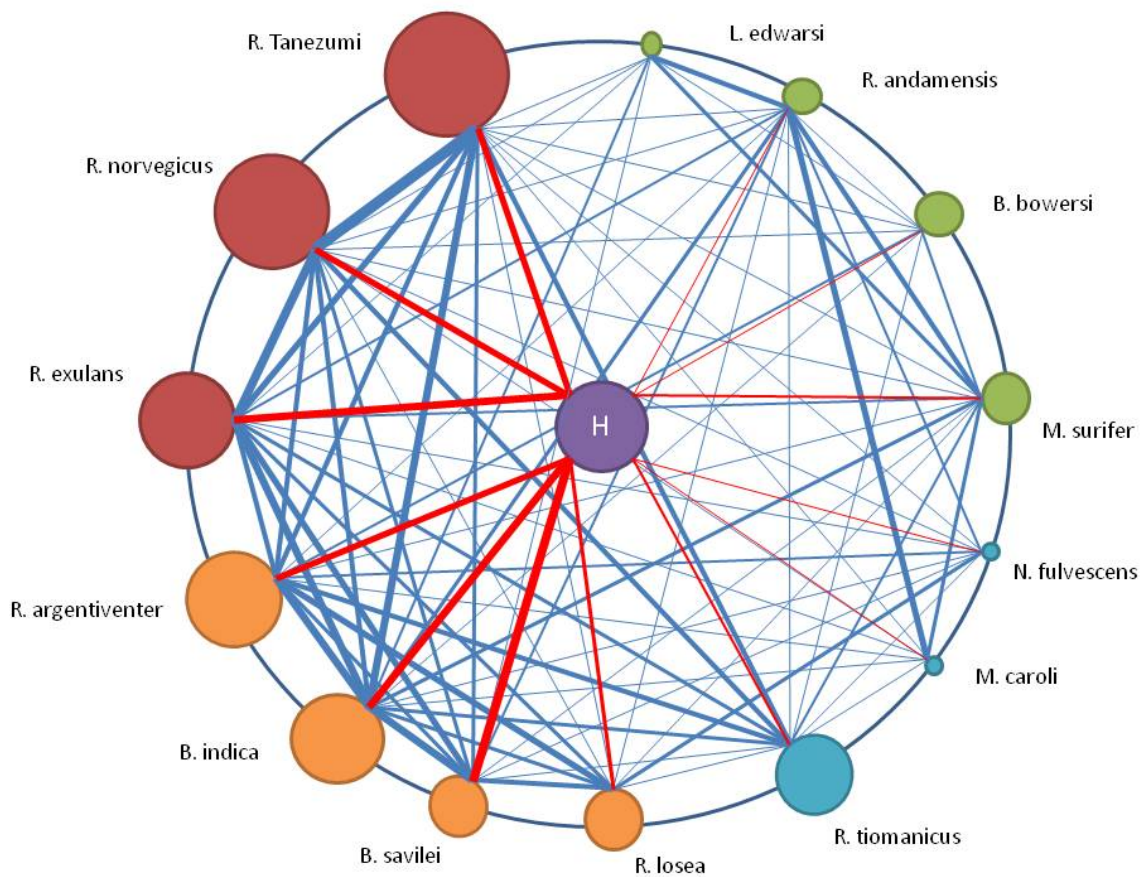
Table A6.1: Host and parasite species used

Target sp.	<i>Homo sapiens</i>
Rodent sp.	<i>Bandicota indicata</i> (Bi), <i>Bandicota savilei</i> (Bs), <i>Verylmys bowersi</i> (Bb), <i>Leopoldamys edwardsi</i> (Le), <i>Maxomys surifer</i> (Ms), <i>Mus caroli</i> (Mc), <i>Niviventer fulvescens</i> (Nf), <i>Rattus andamanensis</i> (Ran), <i>Rattus argentiventer</i> (Rar), <i>Rattus exulans</i> (Re), <i>Rattus losea</i> (Rl), <i>Rattus norvegicus</i> (Rn), <i>Rattus tanezumi</i> (Rta), <i>Rattus tiomanicus</i> (Rti)
Macroparasite sp.	<i>Hymenolepis nana</i> , <i>Rodentolepis</i> sp., <i>Taenia</i> sp., <i>Taenia taeniaeformis</i> , <i>Ascaris</i> sp., <i>Gnathostoma malaysiae</i> , <i>Ganguleterakis spumosa</i> , <i>Citellina levini</i> , <i>Syphacia muris</i> , <i>Physaloptera</i> sp., <i>Rictularia</i> sp., <i>Rictularia tani</i> , <i>Gongylonema neoplasticum</i> , <i>Mastophorus muris</i> , <i>Protospiura-Mastophorus</i> sp., <i>Cyclodontostomum purvisi</i> , <i>Strongyloides ratti</i> , <i>Strongyloides</i> sp., <i>Nippostrongylus brasillensis</i> , <i>Nippostrongylus</i> sp., <i>Orientostrongylus tenorai</i> , <i>Echinostoma ilocanum</i> , <i>Echinostoma malayanum</i> , <i>Notocotylus</i> sp., <i>Quinqueseralis quinqueseralis</i> , <i>Gastrodiscoides hominis</i> , <i>Centrocestus</i> sp.
Microparasite sp.	<i>Leptospira</i> , scrub typhus, <i>Bartonella</i> , hanta virus, herpes virus, LCM virus, <i>Trypanosoma</i> , rabies virus.

Table A6.2: Matrix of Jaccard index for all pairs of host populations; habitat type for rodent species is mentioned: 1=in human settlements; 2=in rice fields; 3=in modified forest and dry agricultural areas; 4=in primary forest; the number of parasite species (Nb of para) per host is also indicated.

	Nb Para	Bi	Bs	Bb	Le	Ms	Mc	Nf	Ran	Rar	Re	Rl	Rn	Rta	Rti	Hs
Bi	16															
Bs	9	0,47														
Bb	7	0,15	0,00													
Le	3	0,12	0,09	0,11												
Ms	8	0,20	0,06	0,15	0,22											
Mc	2	0,06	0,00	0,00	0,00	0,25										
Nf	2	0,06	0,10	0,00	0,00	0,00	0,00									
Ran	5	0,24	0,17	0,09	0,33	0,30	0,40	0,17								
Rar	17	0,43	0,24	0,14	0,00	0,09	0,06	0,12	0,10							
Re	18	0,48	0,35	0,00	0,11	0,13	0,05	0,05	0,15	0,30						
Rl	9	0,19	0,29	0,00	0,00	0,06	0,00	0,22	0,08	0,37	0,23					
Rn	23	0,34	0,23	0,03	0,04	0,07	0,04	0,09	0,08	0,33	0,52	0,19				
Rta	32	0,45	0,24	0,11	0,06	0,14	0,03	0,06	0,09	0,32	0,47	0,17	0,72			
Rti	13	0,26	0,05	0,05	0,00	0,17	0,07	0,07	0,06	0,36	0,24	0,10	0,33	0,36		
Hs	15	0,48	0,50	0,05	0,06	0,21	0,06	0,06	0,18	0,28	0,43	0,20	0,41	0,42	0,17	

Figure A6.3: *Epidemiological Interaction Network for 14 rodent species and the human species in the Southeast Asian ecosystems based on presence-absence data for 34 macroparasite species and 8 microparasite species. Each node represents a host species, the size of the node is proportional to the number of parasite species harbored by the host and the color of the circle represents the habitat in which the host species is mostly found (except for human): red=in human settlements; orange=in rice fields; blue=in modified forest and dry agricultural areas; green=in primary forest. Each edge between two nodes represents the shared parasite community and its width is proportional to the Jaccard index. We placed the human species in the centre of the figure and its edges in red for visual comfort.*



The analysis of this network leads to the following observations:

- Interpreting the size of the nodes, the three rodent species with the highest parasite diversity are occurring in human settlements. The three rodent species with the lowest parasite diversity occur in primary or secondary forests and dry agricultural land.
- The size of the human species node indicates that we share 15 parasite species with rodent species studied here.
- Interpreting the width of the edges at the network level, there is a higher density of large-width edges on the left of the network, indicating that rodent species in human settlements and rice-fields share a higher proportion of their parasite diversity than rodent species in the remaining habitats.
- Interpreting the width of the edges concerning the human species, Bi and Bs have the highest Jaccard index values (0.48 and 0.5 respectively), followed by Re, Rta and Rn (0.43, 0.42, 0.41 respectively).
- The nodes of Bi, Ran, Rn and Rta have the maximum number of edges (n=14) possible in this network. They all occur in human settlements, rice fields except for Ran occurring in primary forest. The nodes of Bb, Le and Mc have the lowest number of edges in the network (n=9) and they all belong to primary and secondary forest or dry agricultural areas.
- The node of the human species has 13 edges close to the maximum of 14.

The method of calculus of the index needs to be kept in mind: the observation that Bi and Bs share more parasites with the human species than Rta and Rn is wrong. The human species shares more parasite species with Rn and Rta than with Bi and Bs (11, 14, 10, 8 respectively). The difference is due to the high parasite species richness of Rn and Rta. Other indices can be used to address this kind of issue but no index is perfect. The highest intensities

of EI are found in rodent species present in heavily disturbed habitat (human settlement and rice fields) and the highest intensities of EI linking the human species with rodent species are also found with rodent species living in these same habitats. This observation suggests that humans are more at risk of contracting new pathogens from rodents present in the human settlement compared to rodents present in primary forest. The availability and patchiness of resources in human modified environment could explain the higher EI intensity in rodent species living in these environments.

This first network can orientate surveillance protocols towards the most interesting host species to be included in order to answer the question at stake: if the question is the probability of EID from rodent host in this ecosystem, the surveillance protocol will target species living in the human settlements (and a ranking can be done on this species) and in the rice fields with maybe *Rti* being an interesting sentinel species to look at as a bridge species between pristine and modified environment. To our knowledge, this species is never mentioned as a potential source of infectious disease or as a sentinel in the literature. If one is more interested in the potential emergence of pathogens in rodent species, *Ran* which shares parasite species with all the other rodent species and humans and lives mostly in primary forests should be considered as a potential source.

In several practical situations, scientists and managers will be concerned by the possible emergence of a specific pathogen in a given ecosystem and for a specific target species. In that case, they should target closely related pathogens or some with similar behaviour (transmission pathways, host range) in order to delineate a more specific EI network. This step can be done by creating a sub-network with the selected pathogens or by weighting the influence of some pathogens in the network. Other *a priori* conditions could be a research question targeting a type of emerging pathogen to look for (e.g. haemorrhagic fevers). For example, several authors have argued that future EIDs will concern in majority RNA virus-type pathogens, due to inherent viral characteristics such as high mutation rate (Poss et al. 2002, Holmes and Rambaut 2004, Cleaveland et al. 2007). The design of a research framework focusing on emerging RNA viruses will select pathogens with similar mode of transmission (other RNA viruses for example) which should use the same EIs. This could be done by focusing the EI network with information from parasites phylogenetically related to the potentially emerging parasite of interest (e.g. separating viruses, bacteria, macroparasites, etc.) and/or sharing the same mode(s) of transmission. The later seems to bear more power than the former, but this will need to be tested. This approach is process orientated and the modes of transmission of parasite will be crucial in determining the transmission pathways between host populations. Groups of parasite with direct, environmental or vector-born transmission could *a priori* produce different EI networks. Three outcomes emerge from empirical data: 1) each EI network is specific for each parasite species and the EI network approach is somehow useless; 2) they are functional groups of parasite sharing a similar EI network (e.g. based on their modes of transmission); 3) EI network are general and produce a transmission framework for all parasite species given that the network is fed with data from enough parasite species. In the case study of Box A6.1, sub-

network could be explored comparing macro- and microparasites EI network or regrouping parasite by modes of transmission and comparing the corresponding sub-network.

Another argument in favour of the inclusion of all types of parasites in the building of EI network using the *a posteriori* approach is that there is no independence between the parasite data from various hosts (Jolles et al. 2006). Each parasite species is in struggle with the host immunity system and in ecological interaction (e.g. direct competition, synergy or indirect through the host immune system) with the other parasites. The community of parasites in the ecosystem will have its own ecological interactions defined by host and parasite presence and densities (Booth and Dunne 2004, Bordes and Morand 2009). Despite some interesting recent results (Graham 2008, Jolles et al. 2008), co-infections of parasites in host populations have not been much explored. The patterns of co- occurrence or exclusion between parasites in hosts are *de facto* included in EI networks and will not create issues for their interpretation. Developing EI network will provide more information on parasite interaction in hosts.

Host populations in the same ecosystem for long periods of time should share more parasites than host populations which have recently come into contact. Here, “recently” refers to a period of time during which host populations did not have time to exchange most of their parasite species. Evolutionary processes should be close to equilibrium in co-evolved host-pathogen interaction (e.g. for Low Pathogenic Influenza viruses and waterfowl, Webster et al. 1992) and the EI network identified quite robust. As time since hosts have been in contact increases, potential transmission events with low probability will statistically increase. In the EID hotspots considered, the time since first contact between a target and a non-target population should be small: human and great apes due to hunting or tourism; livestock and wild ungulates in pristine areas recently colonised; intensive poultry production units recently installed in proximity of wetlands used by wild birds. In EIDs hotspots, by definition,

(human-induced) changes disturb ecological interactions in communities. In this context, the EI identified could be instable (Alitzer et al. 2003, Thomson 2005) and prone to change leading to an evolving EI network. In this context, the *a priori* approach, using host movements and contacts could be used. The *a posteriori* approach will be more difficult to implement in extremely recent interface. However, a few years should be enough for the shared community of parasites to reach a relative equilibrium.

More information could be added in the EI network presented in Box A6.1. First, some of the parasites used, mainly microparasites such as *Leptospira* or Hanta viruses, have benefited from molecular tools able to track minute changes in their genetic load. As mentioned already microparasites are fast evolving organisms compared to their hosts (Poss et al. 2002, Holmes and Rambaut 2004). This can be useful in tracking the origin of infection between different populations, capturing the history of spill-over process at a fine scale. A phylogenetic tree of the parasites strains detected in different host populations could bring information on the directionality of some EI and indicates rodent species as source or reservoir for the rest of host community (for an example see Cottam et al. 2008). Secondly, data from the host population, as presented previously, such as home range or telemetry data could be included to incorporate potential direct and indirect contact between host populations and related to EI identified in the pathogen approach. This would combine the host and pathogen approaches.

Conclusion

After presenting how the use of host data can shed light on transmission pathways between host populations, we developed a multi-pathogen approach, process-centred, to infer epidemiological interaction at the wildlife/livestock/human interface. Theoretical and

technical tools for this approach have already been developed but they are used with a different angle here. Epidemiological data on various pathogens are integrated in a network to predict the behaviour of EIDs, in particular before the emergence event. The reflexion about this approach comes from an empirical point of view, from experiences in areas of limited resources (financial and available data) and tries to answer practical questions of surveillance and control of EIDs where we know that they have a high probability to happen (EID hot spots). The scale of study – the community level – is extremely complex but we suggest that we could benefit from this scale in a resource limited environment.

Albeit empirical, this reflexion links with the modelling approach by concentrating on processes of transmission. The data produced should feed some models (e.g. Arino and van den Driessche 2006) and maybe push the building of models towards including multi-pathogen data to construct EI networks. Network-based modelling can be an entry point with the potential to include social data, facilitating the inclusion of human/animal transmission processes (Perkins et al. 2009, Van Kerkhove et al. 2009).

The context of EIDs in developing countries is an environment where usually little epidemiological data is available except for key diseases, important for government services: if existing data on a pathogen brings light on contacts between two host species, this builds a starting point in the EI network. The gathering of ecological and epidemiological information available for the ecosystem under study, obtained from different sources (e.g. literature, veterinary services, conservation NGOs) can be included in a preliminary EI network which could identify the first surveillance priorities to protect the target species following for example a risk analysis process. The inclusion of the epidemiology of several pathogens in the analysis should explore the results of shared prevalence or strains in relation to the specific modes of transmission. If molecular data exists for a pathogen, the comparison of this data

across host populations can say a lot about the history of contacts between these hosts and about the intensity and the frequency of contacts (Real et al. 2005, Biek et al. 2006).

From a more practical point of view, one could argue that such an approach would be too costly to be funded mainly due to the multiplicity of diagnostic tests which can peak quickly when gene sequencing is needed. The cost of sampling wildlife species in remote areas is high. Specialised team working on particular diseases will be more than willing to collaborate and contribute in terms of laboratory cost in order to access such rare samples. This requires prior communication and agreement with interested teams and logistical arrangements for the right samples to be collected and delivered to laboratories. The accumulation of such collaborations will increase the multi-pathogen data and strengthen the EI network. Multidisciplinary inter- and intra- research team is a prerequisite for this approach.

Finally, if the EI concept survives the test of empirical data, it means that it exists epidemiological “tubes” between different host populations identifying the “highways” of transmission of parasites, ready to be used by the next EIDs. Exploration of this concept can bring new insights in the ecology of disease transmission.

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